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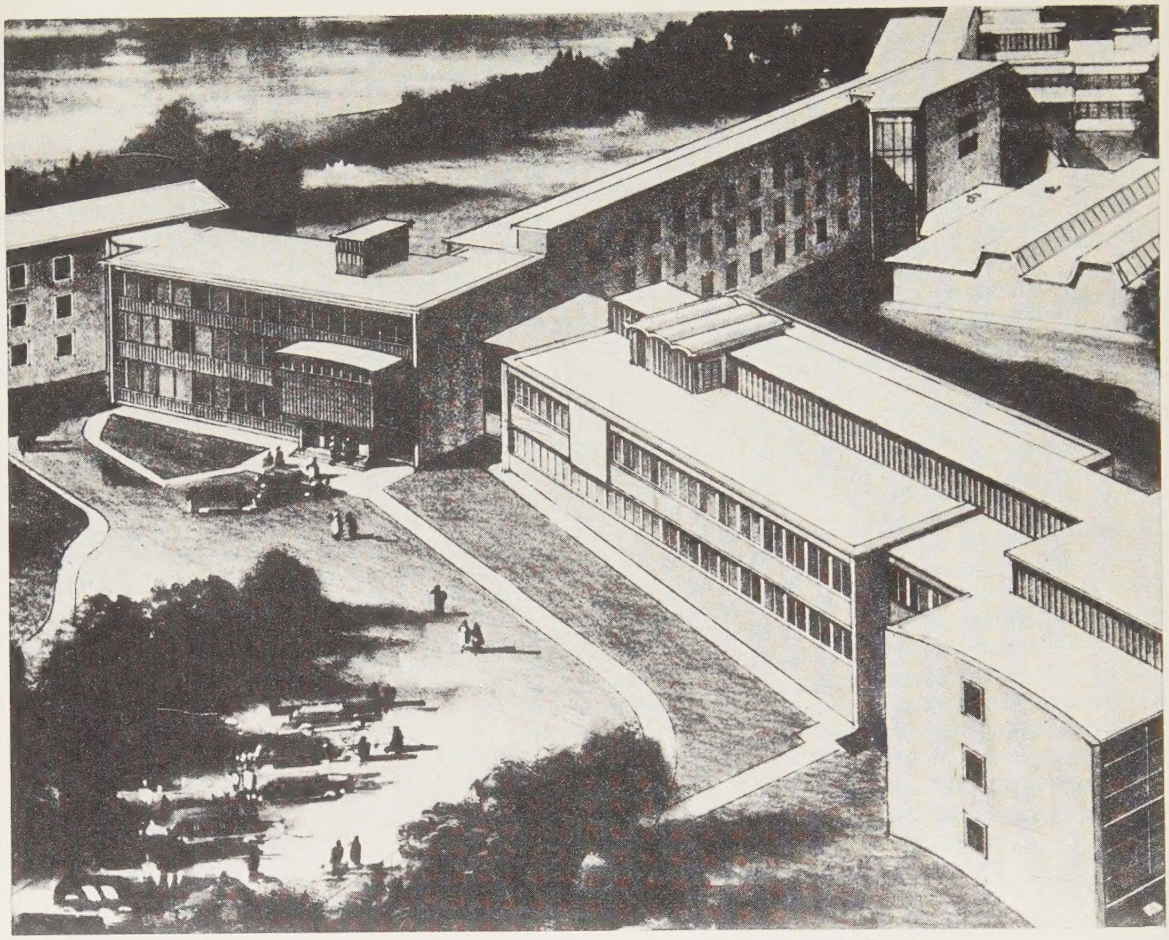

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COMMONWEALTH BUREAU OF ANIMAL HEALTH

VETERINARY REVIEWS AND ANNOTATIONS

Volume 5 **April, 1959** *Part 1*

Scrapie, a Review of the Literature

BY

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Scrapie is known by many names, interesting because they indicate some of the clinical signs seen in the syndrome. In Great Britain the disease is colloquially called Euky Pine, Goggles, Rickets, Trotters, Scratchie, Rubbers, Shakings, Shrew Croft, Cuddie Trot and the Rubs. In France the condition is termed, la Tremblante, Prurigo lombaire, Paraplégie, Raidillon de maladie de nerf, Maladie convulsive, Maladie folle, Maladie nerveuse, Maladie tremblotante, Maladie chancelante, Mal de nerfs, Brandelleuse, Vertigo, Névralgie lombaire, Trembleuse, Névrite périphérique enzootique, Polio-myelo-encephalite à évolution subaigue, Myelo névrite, Névraxite enzootique du mouton. In Germany the condition is known as Traberkrankheit, Gnubberkrankheit, Reiberkrankheit, Wetzkrankheit, Schruckigsein and Kraüselkrankheit.

Scrapie has been recognized in Europe since the middle of the 18th century but it was not until 1898 that creditable investigations into the nature of the disease were attempted, by Besnoit & Morel and by Cassirer. Earlier accounts of scrapie are remarkable for the clarity of clinical description and observations of behaviour, so that throughout the literature the syndrome is easily recognized despite the variety of names given to it.

The first laboratory research was made in France and Germany in 1898. Between 1914 and 1926, interest was aroused in Great Britain by the work of McGowan, M'Fadyean, Gaiger and Stockman, which was directed at the aetiology of the condition. In 1936, Cuillé & Chelle claimed to have transmitted the disease and there followed a succession of

papers on this aspect. Bertrand, Carré & Lucam (1937a) published a full account of the neuropathology.

It is interesting to note that in various countries investigations into scrapie have frequently been instituted by official Agricultural Departments, no doubt as a result of the contemporary severity of the disease; thus, both Bénion and Cassirer addressed their dissertations to their respective Ministries of Agriculture. Similarly, in Great Britain the first authentic account is given in the Journal of the House of Commons (1755) when a petition was presented by the farmers from Boston to restrain 'jobbers' from mixing healthy with 'distempered' animals and reselling them. It appears that these jobbers had a monopoly of the sheep trade. The distemper in question was then called 'rickets' or 'shaking' and many sheep were dying. A special committee was appointed and presented a report, in which the clinical signs were described. On the 28th February 1755, leave was given to bring in a Bill to prevent the spreading of the distemper, but the matter was dropped.

McGowan (1914) gives a full account of the subsequent occurrence of the disease in England, obtaining his information from contemporary agricultural publications. Thus he quotes Comber (1772) as describing stages in its course.

Evidence is also given in McGowan's account about the distribution of the disease: between 1770 and 1815 it was prevalent in Wiltshire, Hampshire, Lincolnshire, Cambridgeshire, Dorset, Somerset, Essex, Kent, Sussex, Norfolk and Suffolk. Throughout many accounts emphasis is placed on the hereditary nature of the disease.

Leopold (quoted by Putz, 1882) recognized the disease in Germany in 1759 and, according to Girard (1829), Tessier gave the first account of it in France in 1810. In 1829, Girard described the clinical signs and divided them into two forms, pruritic and epileptic, or trotting, and many subsequent authors made a similar distinction. Continental writers emphasize the manifestations of ataxia while in Great Britain more notice has been taken of the apparent itch. Roche-Lubin (1848) was particularly interested in the aetiology of the disease and came to the conclusion that among other causes could be counted sexual excess and thunderstorms.

In the mean time, work had been going on in Germany. Some of the earliest evidence comes from an article by Fink (cited by McGowan, 1914) to the English Board of Agriculture in 1804, being a report about the disease in Germany at that time. Contributions between 1810 and 1830 were abstracted by Senac (1827, 1829 and 1831); these reports consist largely of an account of an outbreak on a Prussian State Farm. May (1868) summarizes the earlier work, mentioning that Thaer, Kanert, Ernst, Störig, Spinola and Funke had all failed in their attempts to transmit it, as did Dammann in 1869, who used blood and material from the nasal discharge from affected animals and observed the inoculated sheep for 9–22 months. A full account of the work in Germany is given by Weihermüller (1939).

In 1898, Cassirer made the first neurological investigation of sheep affected with scrapie. He kept 5 animals under close observation and compared their behaviour with normal animals. He found no changes in superficial or deep reflexes, complete paralysis was never seen and the

electrical excitability of the ischiatic nerve remained normal. No significant microscopic changes in the central nervous system (c.n.s.) were observed. Besnoit & Morel, however, published in the same year their neuropathological findings. Chromatolysis and vacuolation were seen in the anterior horn cells of the spinal cord, and an intense neuritis was reported involving the smaller motor nerves. They called the disease 'Névrite périphérique enzootique du mouton'. The animals they used were taken in the last stages of illness. The following year they described the clinical manifestations, suggesting that the paresis affected the extensor muscles more than the flexors; they denied that scrapie existed in two forms; they failed to transmit it.

Because of the apparent sensory disturbance, Friedberger & Fröhner (1905) suggested that there might be some similarity between tabes dorsalis and scrapie, but as they found no lesion in the cord the comparison 'rests upon pure hypothesis, though it has some ground of probability'. Gutknecht (1909) thought that the clinical picture was not so characteristic as previously believed and that the apparent itch was not a constant feature. In 1912, Quevedo described a condition like scrapie in horses, sheep and cattle in Argentina. In 1913, Stockman gave an historical account of the disease in Great Britain, emphasizing its occurrence with the introduction of Merino sheep from the Continent particularly at the end of the 18th century. Other observers have associated scrapie with Merino sheep, but Girard believed 'la tremblante' to have occurred in France before their introduction. Moreover Girard suggested that when Merino sheep were imported more interest was shown in breeding for improved stock: perhaps farmers became more disease-conscious and identified scrapie as an entity for the first time. It has never been seen in Merinos in Australia, though Seddon (1952) reported an outbreak in imported Suffolk sheep which was eradicated by slaughter.

In 1913, Titze distinguished scrapie from louping-ill and in 1914 McGowan published his monograph on the disease, the result of 18 months' investigation of field and laboratory material, carried out for the East of Scotland College of Agriculture. He examined 19 diseased sheep clinically, some for periods of a few days and others for a month or more. No changes were found in the blood picture or in urine; cultures from the cerebrospinal fluid (c.s.f.) were sterile. No changes were found in the c.n.s. and the neurological examination led him to believe that the site of the disease was in the muscles. Histologically, these were found to be heavily infested with sarcocysts and as the active principle they contained, sarcocystin, produced itching and death when injected into rabbits, he concluded that scrapie was due to a heavy infection of sarcosporidia. It should be noted that controlled experiments using muscle extracts free from sarcocysts had no effect on the rabbits, but these experiments were only conducted on 3 animals.

M'Fadyean (1918) was quick to challenge the sarcosporidia theory, pointing out that these parasites are ubiquitous and occur in areas where scrapie had died out. He contended that the disease was infectious, although his own transmission experiments had given negative results.

In 1919 Bigoteau suggested that scrapie was due to infection with Corynebacterium ovis, basing his theory on the evidence of post-mortem

lesions; he was, however, unable to reproduce clinical signs of scrapie by injecting sheep with pure cultures of C. ovis. Carré (1923) doubted the validity of Bigoteau's theory because he found that the intrapalpebral inoculation of C. ovis toxin caused no reaction in animals affected with scrapie but did in those animals suffering from caseous lymphadenitis. Gaiger (1924) tried to produce active immunity to scrapie in sheep by using a carbolized heavy emulsion of brain and cord from affected animals, but without success. He gave evidence that the disease could be introduced into a flock by both rams and ewes and he gave an instance where the entire progeny of a tainted ram, 45 in number, developed the disease. He believed the waves of infection were probably due to the dispersal of badly tainted flocks. G. Moussu (1923) was unable to transmit the disease, using injections of blood and c.s.f. (no mention is made of how long the animals were kept).

Stockman (1926) published the first of a series of papers about his experiments with scrapie, during the previous 12 years; his early death, the same year, unfortunately brought to an end the publication of his observations. Stockman, while at Weybridge, conducted on affected animals some 100 post-mortem examinations and also other diverse investigations. He suggested that scrapie was not hereditary but rather a congenital infection. He doubted whether pregnancy or parturition were important factors in precipitating the disease and thought it unjustifiable to distinguish between nervous and itching forms. No macroscopic lesions having definite connexion with the disease were found, but he substantiated Besnoit & Morel's evidence of the presence of vacuolated cells in the medulla and cord. Moreover, he discovered peculiarly staining bodies, of varying sizes, in the neurones of the cord and medulla which were stained by a variety of techniques. Stockman did not consider these to be specific to scrapie. He found that extracts from sarcosporidia had no effect when injected into normal and diseased animals. He made many unsuccessful attempts to cure the condition, particularly with arsenicals. He also reported one case of spontaneous recovery.

In 1928, French workers again started investigations. Verdier (thesis quoted by Bertrand et al., 1937b) thought that 'la tremblante' was due to a virus, but special soil was necessary to create such 'hereditary' conditions needed for the virus to become infective. Cuillé & Chelle in a series of papers (1936, 1938 and 1939) claimed to have transmitted the disease to sheep by intra-ocular inoculation of an emulsion of lumbar cord taken from affected sheep: 2 sheep out of 9 contracted scrapie after a period of 14 and 22 months — no mention is made of the conditions under which the sheep were kept. The agent appeared to pass an L3 Chamberland filter. A second passage was effected; 4 out of 8 animals succumbed after an incubation period of 1–2 years. Subcutaneous, intra-ocular, epidural and intracerebral routes were all effective. Cuillé & Chelle suggested that the reason for previous failures in transmission was that insufficient time had been given for the disease to develop. They submitted that scrapie was contagious and in 1939 claimed to have transmitted it to goats after an incubation period of over 2 years; these animals exhibited signs of incoordination, fear or trembling, but no apparent itching. An account of a naturally affected goat was given by Chelle in

1942. In 1938, Cuillé, Darraspen, Chelle & Florio described choroiditis and detachment of the retina in animals affected with the disease. This observation was not supported by pathological evidence.

At much the same time, 1937, Bertrand, Carré & Lucam attempted to transmit the disease, but did not succeed, even though they used various routes and observed their animals for up to 23 months. These workers also made a detailed study of the neuropathology. Neurones in various stages of degeneration, including vacuolation, were found in the cord, medulla, pons, and midbrain. There was an astrocytic reaction and changes in the neurofibrils, but no inclusion bodies or evidence of a peripheral neuritis were seen.

During the period 1935–40 Brownlee made several observations about the disease; he found demodex mange mites in the sebaceous glands of the skin in affected animals but these were a common parasite in healthy animals and scrapie could occur in their absence. He also made a histopathological study of the c.n.s. and found vacuolated cells in the medulla and/or spinal cord, but none in his group of 64 controls except for 'a few cases, the majority of which were sheep infected with louping-ill virus'. The vacuoles in the latter condition, however, differed from those seen in the neurones of sheep affected with scrapie, in that they were much smaller and occurred only in necrotic cells, whereas in scrapie the cells containing the vacuoles appeared normal apart from vacuolation. No changes were found in other organs except in one sheep where there were 'extensive areas of coagulation necrosis' of parts of the adrenal medulla. In contrast Weihermüller described degenerative changes in the thyroid, an increase of the cellular parts of the anterior lobe of the pituitary, an increase in the size of the zona reticularis of the adrenal cortex but a decrease of the zona fasciculata. Weihermüller did not consider these changes to be constant in scrapie.

In 1938 Schofield reported the first outbreak in North America.

Except for the work of Holman & Pattison, Wilson, Anderson & Smith, Greig, Gordon, and Wagner, Goldstein, Doran & Hay, there have been no significant investigations between 1946 and 1956; but there have been fresh outbreaks of scrapie especially in Canada, the U.S.A., Australia and New Zealand.

Holman & Pattison (1943) described the neuro-anatomical arrangement of the medulla of the sheep and showed that the distribution of vacuolated cells in animals affected with scrapie was selective for certain nuclear groups, especially the reticular formation, the medial vestibular and lateral cuneate nuclei. Moreover, in 75 affected sheep, all showed vacuolated nerve cells in the medulla and these abnormalities were rarely found in animals dying from other conditions. Statistical examination of these results indicated that vacuoles were to be expected in not less than 94% of cases. They also described homogeneous granules within the vacuoles that stained pink with eosin. Holman (1945) made a survey of the blood picture of sheep, in relation to various diseases; he concluded that in scrapie there was eosinophilia during the incubation period and eosinopenia with neutrophilia up to 10 days before death.

Wagner et al. (1954) began a study of scrapie in Ohio. In their first communication they described the clinical phenomena and neuropathology,

the latter in their opinion consisting of vacuolation of neurones, a slight suggestion of gliosis, variable degeneration of Purkinje cells and the presence of intracytoplasmic and intranuclear inclusion bodies.

Research in Great Britain on scrapie has been concentrated since the war at the Moredun Institute, Edinburgh and although little has been published, much has been done. Interest was roused in 1937 (Gordon, 1946) when scrapie occurred in flocks which two and a half years before had been inoculated with louping-ill vaccine. Scrapie appeared in those animals vaccinated with a certain batch (batch II) of material and not in those vaccinated with two other samples; all the vaccine had been prepared by the same method, using a formolized suspension of brain, spinal cord and spleen of sheep infected with louping-ill. Eventually, it was realized that eight of the sheep which had been used for the suspected vaccine had in fact been the progeny of ewes which had been in contact with scrapie, some of the ewes actually developing the disease later. Of the 18,000 sheep vaccinated with batch II, between 1 and 35% developed scrapie. Total figures are not available because the older animals, inoculated in 1935, were disposed of before 1937.

The results of this accidental experiment indicated:-
(1) the infective agent of scrapie was present in the brain, spinal cord and/or spleen of infected sheep; (2) the agent could withstand concentrations of 0.35% formalin, which inactivated louping-ill virus; (3) the transmission occurred through subcutaneous inoculation; (4) the incubation period was two years or longer.

In 1938 (see Gordon) an experiment was set up with 788 animals to try to confirm these results. After four and a half years, 60% of those inoculated intracerebrally and 30% of those inoculated subcutaneously became clinically affected. Intracerebral inoculation resulted in a shorter incubation period (7 months) than the other route (15 months).

In confirmation of Cuillé & Chelle's experiences it therefore appeared that scrapie could be transmitted with material obtained from the c.n.s. and spleen of affected animals.

Greig (1940a) reported that Dryerre & Yule Bogue had shown that the metabolic rate remained relatively unaffected throughout the disease. Greig also wondered whether contamination of pasture would result in spreading the disease; he set up an experiment with 26 sheep from a 'clean' farm, introducing affected sheep to the pasture, twice a week for three years. Care was taken that there was no immediate contact between the experimental and affected animals. After three years, the former were removed to another clean farm and in the following two years, seven animals died from scrapie (confirmed by the presence of vacuolated neurones).

This finding suggests that contamination of pasture can lead to spread of the disease and illustrates some of the difficulties which arise when working with this disease. It is quite impossible to be certain that 'clean' animals are not indeed carrying 'infection' because (1) of the extremely long incubation period, and (2) there is no test that can detect scrapie in animals before clinical signs are evident. Moreover, if indeed Greig did transmit scrapie by pasture contamination (assuming that in fact the affected animals never came in contact with the experimental sheep –

quite a feat in flock management), it is almost impossible to imagine conditions under which transmission experiments can be carried out without the constant risk of a similar contamination of pasture or fodder.

Wilson et al. continued these experiments in 1945, attempting to get over the difficulties by injecting material from affected sheep into normal lambs and discarding any of the latter which did not develop symptoms within a year, as he regarded the average incubation period to be six months. He succeeded in transmitting the disease to 21 of 83 seven-month-old Cheviot wethers, which developed symptoms within 5–10 months. He used ultrafiltrates of pooled c.n.s. material from affected sheep, ultrafiltrates that passed through Gradocol membranes of A.P.D. (not limiting) of 0.65μ and 0.41μ.

As Wilson et al. considered that the agent was a virus and they wished to maintain it, they tried to passage it through sheep; nine passages were made. In all, 33 animals were inoculated intracerebrally and in 17 scrapie developed, although a further six showed transient symptoms. The incubation period was approximately five months. All the animals were under two years old when injected. Once scrapie had developed clinically, some survived for a period of a few days while others lived for up to four and a half months. Vacuolated neurones were found in all cases.

In another experiment, 40 sheep were inoculated with material obtained from two rams killed in the late phase of scrapie; all the transmissions failed. In the earlier passage experiment all the inocula, except one, had been made from the c.n.s. of affected animals taken early in the disease. When the 'infective' agent was subjected to drying and storage at 0–4°C. for periods of over two years, it was still capable of reproducing the disease.

In 1956, Henderson reported an unsuccessful attempt to cure clinical cases of scrapie with massive doses of vitamin B_{12}. Bosanquet, Daniel & Parry (1956a) and Parry (1957) described myopathic changes in sheep affected with scrapie but gave little indication of the number of lesions found in the animals. Bosanquet et al. believed that this myopathy could go a long way in explaining the manifestations of the disease, although similar lesions were also found in sheep unaffected with scrapie. No signs of myopathy were observed in control animals. These authors considered that the vacuolation of neurones, so frequently reported by previous workers, was of little significance.

However, Pattison in 1957 published an account of the presence of vacuolated nerve cells in the medulla of goats, animals which had previously been inoculated with material from affected sheep. This vacuolation in the goat was far more intense than that observed in control animals. Also in 1957, Palmer described in further detail the nature of intravacuolar material in scrapie and recorded the presence of eosinophilic material in the cytoplasm of non-vacuolated neurones in affected animals. But Zlotnik (1957a) also observed this material in the cytoplasm of neurones in healthy sheep.

Zlotnik & Rennie (1957) reported the presence of vacuolated nerve cells in the medulla of apparently normal sheep. In these experiments 57 animals were used, mostly Cheviots. This work was based on the

examination of 180 sections from each medulla. The frequency of occurrence of affected nerve cells varied between sheep; in some, vacuolation was fairly intense (up to seven affected neurones/slide) while in others no affected neurones were found. The occurrence of intra-vacuolar material and perivascular cuffing in apparently normal sheep was also demonstrated. These workers explained the incidence of vacuolated nerve cells in healthy sheep as follows: (1) the sheep were incubating scrapie; (2) the sheep were affected with a non-clinical form of the disease; (3) vacuolation of neurones is not directly connected with scrapie.

When Zlotnik (1957b) came to examine material from field cases of scrapie, he discovered a high incidence of vacuolated neurones but only a few affected cells in a control group of sheep. He concluded that there was a correlation between the vacuolation and scrapie but emphasized the necessity for examining serial sections of the medulla before assessing the significance. He did not regard the occurrence of up to seven vacuolated neurones in an isolated section as being significant.

In a paper given at the 75th Annual Congress of the British Veterinary Association, Palmer (1957) emphasized the importance of the neuropathological changes in scrapie. The morphology of the vacuoles was described and experimental evidence provided to show that the vacuoles are not artefacts. The neuro-anatomical distribution of affected cells (based on the examination of interrupted serial sections of four brains from diseased sheep and three brains from normal sheep) was briefly described (see Palmer, 1958). In affected sheep vacuolated cells were particularly found in the lateral cuneate nucleus, the lateral caudal part of the reticular formation and in the papilliform nucleus; affected regions were usually bilaterally symmetrical. As the result of this experiment, a standard level of the caudal part of the medulla was selected and the frequency of vacuolation was compared between 19 sheep affected with scrapie, 16 normal sheep and 13 sheep with other nervous diseases. There was a much higher incidence of vacuolated neurones in sheep affected with scrapie than in the other two groups. Palmer was unable to find convincing evidence of a glial reaction. In seven sheep and two goats affected with the disease, in which the skeletal musculature was examined, only three muscles from three animals were found showing degeneration similar to that described by Bosanquet, Daniel & Parry.

When opening the discussion of this congress paper, Gordon (1957) reported that, at Compton, Bracewell had examined the muscles of eight natural cases of scrapie without finding any myopathic lesions. Gordon also described the transmission experiments conducted between 1938–42. Indications that the goat provides a more profitable animal than the sheep on which to conduct transmission experiments were given by Gordon & Pattison (1957). Of 67 goats inoculated intracerebrally with material from affected animals, all showed clinical signs of the disease. Moreover the disease was passaged in series, through three groups of goats by the intracerebral route and then passed back to sheep from goats. Of the 20 south Scotland Cheviots inoculated with material from the goats, eight developed signs of scrapie.

The problem whether muscle degeneration is important in scrapie

was investigated by Delez, Gustafson & Luttrell (1957). They examined 20 field cases of the disease and found vacuolated neurones in the brains of 17, especially in the medulla. Equally prominent vacuolation occurred in normal sheep and there appeared to be no essential difference in the frequency of distribution between the normal and affected animals, although numerical evaluation to support this result was not reported. Muscles from three of the diseased animals were examined and muscular dystrophy was regularly found in certain muscles of the hind-legs, extrinsic muscles of the eye and cutaneous muscles of the forehead.

Stamp (1958) described current research at the Moredun Institute. Hulland was unable to demonstrate lesions of polymyositis in affected sheep. Stamp, Brotherston, Zlotnik and Smith inoculated 14 Cheviot lambs, less than a day old, with material from affected sheep: 11 of the lambs developed typical signs of scrapie when three and a half to five months of age. Stamp described unpublished experiments by Wilson, D.R. (1954) in which the transmissible agent survived heating at 100°C. for 20 minutes.

A detailed account of the histopathology of the brain stem of field cases of scrapie was published by Zlotnik (1958), who studied 10 sheep with advanced scrapie, 10 with early signs and 10 unaffected sheep, all animals of comparable ages. Zlotnik described various stages of neuronal degeneration associated with vacuolation and attempted to classify vacuolated neurones morphologically. In normal sheep, the few affected neurones that Zlotnik found had only large vacuoles with no other cell change. In sheep suffering from scrapie, vacuolated cells occurred throughout the medulla, and were usually abundant in the caudal region. There were usually more vacuolated nerve cells in cases of advanced scrapie than in the earlier stages.

In addition to vacuolation Zlotnik gave an account of other changes observed in the medulla of diseased animals. There was perivascular oedema, loose perivascular proliferation of oligodendroglia and microglia and also infiltration of microglial cells into the walls of the capillaries. The media of small arteries was undergoing proliferation and hyalinization and there was some evidence of perivascular haemorrhage. In 70% of the advanced cases of scrapie there was perivascular demyelination and fat-granule cells occurred in perivascular spaces. Astrocytic proliferation was present, particularly in the region of the inferior olive and the dorsal motor nucleus of the vagus. There was evidence of other glial changes including satellitosis and neuronophagia. Occasionally there were abnormalities in the cerebellum including vacuolation, disappearance of Purkinje cells and oedema of the granular layer.

Evidence that the skeletal muscles are not primarily involved in scrapie was reported by Hulland (1958), who made an exhaustive search of the skeletal muscles from 30 cases of scrapie (14 experimentally induced and 16 natural cases) and compared them with muscles from 12 control sheep. Hulland found macroscopic lesions in one natural case and minor microscopic changes in a further five, two natural cases and three experimentally induced. He considered that in scrapie clinical signs could not be ascribed to changes in the muscles.

Palmer (1958) elaborated results reported in 1957, including the

neuro-anatomical distribution of vacuolated neurones in four affected sheep. In addition an illustrated account was given of the cytoarchitecture of the medulla and midbrain of the sheep. Experimental details were reported of an unsuccessful attempt to reproduce clinical signs of scrapie by the introduction of pharmacological agents into the lateral ventricle of the brain in normal sheep.

SUMMARY

Scrapie is a disease of sheep characterized by signs of intense irritation, abnormal gait, hyperexcitability, tremor and debility. The disease can be transmitted from sheep to sheep, from sheep to goats and from goats back to sheep. The incubation period appears to be from three and a half to five months when day-old lambs are inoculated. Transmission of the disease to goats appears to be more successful than transmission to sheep. There is evidence that the transmissible agent is present in the brain and spleen and that the agent can withstand extraordinary chemical and thermal insults. Most workers agree that vacuolated neurones occur in the medulla of affected sheep more abundantly than in healthy animals. Vacuolated cells are found particularly in the caudal part of the medulla and the regional distribution is usually symmetrical. Reports that scrapie is essentially a disease of muscles have not been substantiated.

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Specific Antibodies at Mucous Surfaces

BY

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INTRODUCTION. Modern concepts of antibody formation require the close association of antigen with certain specialized cells of the lymphoid-macrophage system (see Taliaferro, 1949) having a functional though not structural unity, therefore all antibodies at their point of origin may be considered local. Similarly antibodies present in mucin at the surface of a mucous membrane which are frequently described as local, may be derived from sites in the immediate vicinity or from humoral antibody extravasated from the plasma. In the present context the term "local" will be applied only to those antibodies which have apparently originated at sites adjacent to the mucous membrane and which have been liberated in such a manner as to pass in some degree on to the mucous surface. They will be contrasted with humoral antibodies elaborated at sites remote from the mucous surface and to which they have been transported by the circulatory system.

The term muco-antibody is used to describe local or humoral antibodies at a mucous surface although the prefix only implies their admixture with the muco-proteins. Thus muco-antibodies may be local or humoral in origin or both but circulating antibodies while essentially humoral may in part be local in origin.

There is evidence for local resistance to infection which is non-specific although stimulated by pathogens. This aspect will not be considered although it may be of some importance when assessing evidence for the resistance to infection attributed to muco-antibodies. Nevertheless the circumstances under which muco-antibodies are likely to be of significance in the resistance of the host can to some extent be related to the individuality of the invading organism. Pathogens characteristically gain access to the tissues of the host and there in some degree multiply. Multiplication may be localized at the primary site of entry, sometimes with the liberation of exotoxins with profound systemic effects, or in organs characteristic for a given pathogen and far removed from the portal of entry. Others may give rise to a generalized infection of the circulatory system and all three states frequently exist at one time or another for a given pathogen. Muco-antibodies are likely to be of the greatest significance with regard to pathogens which are not invasive, nor liberate powerful exotoxins and which become established at a mucous surface or in the superficial layers of the mucous membrane. In many of these infections the presence and concentration of antibody in the plasma can have little direct significance, nevertheless it is frequently assumed that circulating antibodies detected by a given in vitro test are protective

and that they so distribute themselves through the tissues that organisms at a mucous surface experience concentrations at an effective level. Since this assumption will be shown in many cases to be incorrect it is not surprising that there have been failures to demonstrate a correlation between acquired resistance and circulating antibodies, particularly when these have been stimulated by the usual methods of artificial immunization.

Some of the earliest work directed towards the demonstration of the local production of antibody was by Bursuk (1928). Following the intracorneal injection of rabbits with typhoid bacilli and staphylococci, agglutinins were found in the local tissue earlier and in greater concentration than in the serum. This work was confirmed by Thompson et al. (1936) using crystalline egg albumen; the absence of antibody in the untreated cornea was also demonstrated.

ALIMENTARY TRACT. Cholera, bacillary dysentery, and certain Salmonella infections are confined essentially to the lumen and mucosa of the gut and consequently the formation of specific intestinal muco-antibody — usually referred to as copro-antibody — has been studied in some detail.

Specific copro-antibody in saline extracts prepared from the faeces of patients with dysentery was first demonstrated by Davies in 1922. Copro-agglutinins were detected against Shiga and Flexner bacilli during the first week of infection; considerably earlier than serum agglutinins which are not usually detected before the 6th–12th day of infection (Wilson & Miles, 1955).

Davies demonstrated the diagnostic significance of these antibodies and Russian workers were subsequently responsible for the extension of this method for diagnosis. Predtetchensky & Moroz (1940), Skochko (1942) and Yampol'skii et al. (1944) confirmed the specificity of the reaction and recommended the detection of copro-antibodies for the rapid laboratory diagnosis of dysentery. Positive results were obtained by the 2nd–3rd day of infection and since corresponding serological tests were frequently negative, the copro-agglutinin was considered local in origin (Predtetchensky & Moroz, 1940). Similar results were obtained by Harrison & Banvard (1947), who compared serum and copro-antibody titres at different stages of Shigella sonnei and flexneri and Salmonella infections in man. Copro-agglutinins were detected during the early stages of acute infections, appearing by the 3rd day and rising to a high titre by the 9th day. Soon after the faecal culture was negative the copro-agglutinins disappeared. The serum antibodies reached significant levels at the time of recovery, and thereafter persisted at a high level.

In contradistinction to these observations González et al. (1950 a, b), investigating a mild bacillary dysentery (Shigella paradysenteriae Flexner) in man and monkeys, failed to detect copro-antibody, although serum antibodies were developed. Further experiments on a small number of monkeys (Macaca rhesus) which were given live and formalin-killed Sh. paradysenteriae per os also failed to stimulate demonstrable copro-antibody (González & Koppisch, 1951).

Disappointing results were obtained by Gordon et al. (1950) in studies

on a large number of human subjects given heat-killed Shigella flexneri III parenterally or enteric-coated vaccine per os. The increase in copro-agglutinins was only significant when examined statistically in the group given parenteral Shigella vaccine, oral vaccine gave no significant increase in copro-antibody titre. However, the copro-agglutinins detected were in conformity with other work; early and transitory in appearance, and preceding the period of maximum serum titre.

Barksdale & Ghoda (1951) showed that 95% of 50 sera and 30% of normal faeces from non-dysenteric soldiers contained agglutinins for the H and O antigens of a strain of Escherichia coli. This strain was carefully selected for a broad reactivity with the coli group and for being inagglutinable by standard Shigella or Salmonella sera. Further studies on Shiga, Sonne and Flexner types of dysentery showed a parallel rise and fall of E. coli copro-agglutinins with the agglutinins of the recovered dysentery bacilli. Similarly the E. coli copro-antibody of normal rabbits was elevated following parenteral immunization with a heterologous organism (S. paratyphi A). However, the titre to E. coli was higher in faecal extracts than in the serum while the titre in the serum to the S. paratyphi was 1:5,000 compared with a maximum copro-antibody titre of 1:125. These authors conclude that a rise in copro-antibody could be misleading for the diagnosis of salmonella or shigella infections since antigenic components could be shared with E. coli. A case of chronic ulcerative colitis is recorded in which copro-agglutinins against several species of Salmonella and Shigella were demonstrated with the bacteriological demonstration of only a coliform bacillus which may have been responsible (Barksdale et al., 1951).

These results demonstrate the presence of specific antibodies in the faeces and suggest that at least in part they may be local in origin, however a careful and comprehensive experimental approach to the study of the copro-antibodies has been carried out by Burrows and his colleagues, using infections of Vibrio cholerae in the guinea-pig. The organism gains access to the small intestine per os and the infection is confined to the lumen of the gut; it is improbable that the invasion of the deeper tissues of the intestine plays an important role in the pathogenesis of the disease. Burrows et al. (1947) showed that either previous infection or the intraperitoneal injection of dead vaccine protected guinea-pigs from further infections. The copro-antibodies demonstrated following intraperitoneal vaccine, may have been local or humoral in origin according to the distribution of the antigen. Since normal and infected faeces contained protein insoluble in half-saturated ammonium sulphate solution some globulin was apparently a normal constituent of faeces and was capable of passing the intact intestinal mucosa. Since the copro-antibodies stimulated by intraperitoneal immunization were less persistent than those in the serum, it was possible to show that, following the disappearance of copro-antibody, guinea-pigs became fully susceptible despite the persistence of appreciable serum titres. This important observation showed that the resistance to infection was related to the muco-antibodies rather than to the circulating antibodies. A generalized infection in guinea-pigs by V. cholerae following the inhibition of copro-antibody by large doses of X-irradiation also suggests the protective role

of copro-antibodies (Burrows, Deupree & Moore, 1950 a, b). Burrows & Havens (1948) immunized human volunteers with cholera vaccine parenterally and per os by ingestion in enteric-coated capsules. Antibodies developed in the urine and faeces before they could be detected in the circulation and declined during the period of peak serum titres.

Guinea-pigs passively immunized by the intraperitoneal injection of homologous and heterologous (rabbit) antiserum developed copro-antibody and urinary antibody while serum titres, as in actively immunized guinea-pigs, were not detected until after the peak copro-antibody titres. (These results are discussed below, under Koshland, 1953.)

Koshland & Burrows (1950), in a quantitative study of the response in guinea-pigs to intraperitoneal vibrio vaccine, considered that the copro-antibody represented a measure of the rate of local antibody formation while the serum antibody represented the accumulation of antibody.

Burrows & Ware (1953) studied the active and passive immunity acquired by guinea-pigs to V. cholerae by measuring the increase in the I. D. 50 compared with normal susceptible controls. The prophylactic immunity was not a simple one related to the titre of the copro-antibody but seemed to be raised when the antibody, whether actively or passively acquired, was being secreted into the bowel. For example although the titre in the intestine was high following the intragastric inoculation of antibody, less protection was afforded against immediate infection than 14 days later when the absorbed passive antibody was being secreted again into the bowel. The interpretation of these results is difficult and suggests some protective activity not quantitatively revealed by agglutinin titres. Complement activity with the lysis of the vibrio following the secretion rather than the local application of antibody did not appear to be a significant factor as faeces were shown to be strongly anticomplementary. Burrows (1953) showed that the vibrio was capable of reducing the agglutinin titre by specific absorption, and the viable counts of organisms in faeces could be reduced by 70–80% following the addition of antiserum. Thus the intraperitoneal injection of immune serum which would have produced a copro-antibody titre in normal guinea-pigs did not do so when the guinea-pigs were already infected, but the copro-antibody titre appeared later with the disappearance of the vibrio and was partly an active immune response to the infection.

The evidence presented by Burrows et al. in support of a local origin for copro-antibody is supported by Koshland (1953). The injection of cholera vaccine in conjunction with an adjuvant stimulated a high serum titre in the guinea-pig without the concomitant copro-antibody titres. The circulating titres were in some cases higher than those produced in guinea-pigs by Burrows using vaccine but no adjuvant and in which copro-antibodies were detected. Koshland explains the absence of copro-antibody in experiments using adjuvant by postulating the immobilization of the antigen so that none or insufficient amounts reach the local sites of copro-antibody formation. These results strongly support the local origin of copro-antibody and its derivation from local cells of the lymphoid-macrophage system.

Antiserum, prepared by the injection of cholera vaccine and adjuvant,

injected into the peritoneal cavity of guinea-pigs passed passively into the gut and was detected in the faeces in conformity with Burrows' observations. Since humoral antibody when actively produced did not contribute extensively to the copro-antibody, Koshland suggests that in this case antibodies gained access to the lumen extra-vascularly.

Helminths which parasitize the lumen of the gut might be susceptible to copro-antibodies, nevertheless the literature has yielded no evidence of a direct approach to the demonstration of muco-antibodies against helminths.

In the absence of direct evidence of copro-antibody against helminths some indication of a local immunity within the gut would be most convincingly shown by the effect of passive immunization on the intestinal phases of helminth infection.

Sarles & Taliaferro (1936) and Taliaferro & Sarles (1939) showed that rats infected with Nippostrongylus muris acquired an immunity which could be passively transferred. This was evidenced by stunting and by a reduction in egg-laying capacity, of those worms reaching the intestine. The interference with infection rather than the absolute death of the parasite is a typical manifestation of helminth immunity which may be confusing to those more familiar with the immune mechanisms associated with acquired resistance to bacterial infections. Females began to lay eggs 6–7 days after infection, nevertheless in two weeks an active immunity was acquired and the adult worms were expelled from the intestine. Whether the antibodies responsible for this immunity are local, humoral, or both, in origin has not been determined and only the immunity has been demonstrated. However, the immune environment has been shown by the growth and egg production (Chandler, 1936a) of stunted worms when transplanted into the intestines of non-immune animals.

There has been much discussion concerning local as opposed to humoral immunity to helminth infection (see Chandler, 1935, 1936b, 1937, 1939 and Taliaferro, 1940). However, Taliaferro & Sarles (1939) suggest that the situation may be complicated by the feeding habits of the adult helminth, which involve piercing the epithelium, so that circulating antibodies may be transmitted to the parasite; moreover they have shown that the living helminth is not susceptible to antibody through its cuticle.

The work by Burrows et al. and Koshland (1953) already outlined, suggests that antiserum when given intraperitoneally may not give results with regard to the recovery of copro-antibody coincidental with those resulting from the active stimulation of essentially humoral antibodies. Therefore it may be significant that many examples of the successful transfer of a passive immunity against helminths in the gut have been obtained following the intraperitoneal injection of antisera (Sarles & Taliaferro, 1936; Chandler, 1938; Sarles, 1939) and the results would not necessarily indicate the availability of circulating antibody to the lumen of the gut. On the other hand the passive immunization of rats by Sarles (1939), when the antiserum was given intravenously, is of particular interest. The antiserum was injected on the 5th day of infection with N. muris by which time the helminth had completed the migratory phase, reached the intestine and largely completed development; the worms were eliminated from the intestine. While this result does not preclude local

antibody formation it does show that circulating antibodies were effective against intestinal phases of this helminth infection. Whether inflammatory changes due to the infection or perhaps metabolic products of the helminth may induce changes in permeability of the vascular system with a resultant extravasation of antibody has not been investigated, however, the feeding habits of this parasite already referred to may provide most effective access of antibody to the parasite.

Sarles (1939) showed that in both active and passive immunization the immunity to N. muris was most evident in the intestinal phase, and a similar intestinal immunity has been noted with Trichinella spiralis (Ducas, 1921; McCoy, 1931; Bachman & González, 1936). McCoy (1936) observed that in rats with an intestinal immunity to a challenge infection with T. spiralis, the development of trichinella larvae placed in the pregnant uterus was not inhibited. Culbertson & Kaplan (1938) demonstrated in mice the transference of a passive immunity against the intestinal phases of T. spiralis in that only half as many larvae developed to maturity as compared with fully susceptible mice; however, although these authors visualized an immunity conferred via the circulation, since the serum was given intraperitoneally once again the antibody could have been conveyed by a non-vascular route to the lumen of the gut. Stoll (1929), in studies on Haemonchus contortus infection in sheep, showed that an existing worm burden was rapidly eliminated following a superimposed dose of infective larvae. This self-cure phenomenon has not been studied in relation to muco-antibodies, nevertheless Stewart (1950) and Stewart & Gordon (1953) showed that sheep infested with Trichostrongylus spp. and T. colubriformis acquired a resistance which appeared to be immunological in character. Although the self-cure phenomenon was associated with a rise in circulating antibodies Stewart (1953) showed that the increase in titre followed the self-cure. This suggested that the circulating antibody per se was not effective and this was confirmed in further experiments which showed the inhibition of self-cure by antihistamines without the concomitant inhibition of the rise in circulating antibody. Interest was therefore focused on the local site of infection and Stewart (1953) found histologically an oedema of the mucous membrane when administration of live larvae had set off the self-cure phenomenon. It was of particular interest that the zone of reaction was specific to the area of infestation: Haemonchus contortus produced oedematous changes in the abomasum only, of sheep infected only with H. contortus; and in the small intestine only, of sheep infected only with Trichostrongylus spp. It was unlikely therefore that the sensitivity was conferred by humoral antibody; the essential feature would appear to be a local hypersensitivity in which the reaction was elicited by the sudden acquisition of a relatively large amount of specific antigen. Research directed towards the study of local cellular changes resulting from helminth infection has revealed lymphoid infiltration. Interest has been focused on globule leucocytes with regard to local concentrations of antibody by White (1954) using the Coons, Leduc & Connolly (1953) fluorescence technique, and these cells have been shown by Sommerville (1956) to be present in the abomasal mucosa of sheep parasitized with helminths for more than 35 days, and absent (with one exception) from worm-free sheep and sheep infested for 35 days or less.

UDDER. Smith, Orcutt & Little (1923) studied the origin of agglutinins to Brucella abortus present in cows' milk. Comparing the ratios of milk titres to blood titres, two groups were evident, one fluctuating between 1:16-1:64 and the other from 1:1–1:4. These groups did not include animals secreting colostrum; here the ratio was 2:1 and declined a few days after calving to 1:16–1:64. A possible explanation for the two groups was derived from the results obtained from two animals injected subcutaneously with living Br. abortus. Both cows were at a similar stage of lactation and the blood titres evoked were similar. However, one animal was secreting large numbers of brucella in the milk and came within the 1:4–1:1 ratio group while in the other no brucella were recoverable from the milk and the animal came within the 1:16–1:64 group. Since the serum titre was always higher than the milk titre these results could indicate local antibody production or an alteration in tissue permeability dependent upon local infection; the latter possibility will be referred to again and has been described as "pathotopic potentiation". The subcutaneous or intravenous injection of immune serum produced only a very slight rise in milk titre, so that in the normal secreting udder at this period of the lactation cycle there was an effective barrier between circulating antibody and the milk. When dead antigen was injected into the udder high local titres were obtained although not as high as in the blood. A local inflammatory reaction was evident in the injected quarter so that, although the animal fell into the low ratio group, again altered tissue permeability and not local antibody formation could have been responsible.

Blakemore (1949, 1951) considered that local antibodies were not formed following the infusion of one quarter of the bovine udder with killed Br. abortus. Instead there was a rise in blood titre not reflected in the udder until seven days after the circulating antibodies had appeared, and then agglutinins appeared simultaneously in all four quarters.

Observations by Balozet (1947) showed that the Brucella agglutinin titre in milk obtained from the half of a goat's udder with mastitis was several times higher than that in milk from the normal half. In the absence of a local brucella infection in the inflamed half, these results would indicate local alteration in vascular permeability. A permeability factor was also suggested by Kerr, Pearson & Rankin (1958) as an explanation for the rather transient occurrence of Br. abortus agglutinins in whey following subcutaneous vaccination with Br. abortus Strain 19 living vaccine. However, the use of live vaccine may be criticized where unequivocal evidence is required with regard to the site of localization of antigen. Although Kerr et al. were unable to recover viable organisms from the milk this does not preclude possible local antigenic stimulation particularly when antibodies to other organisms which were present in the circulation were not detected together with the brucella agglutinins in the milk.

Mitchell, Walker & Bannister (1953a,b, 1954, 1956) showed that influenza A and Newcastle disease virus instilled into the bovine mammary gland propagated for several days and stimulated neutralizing antibodies which appeared to be local in origin. They were detected in milk from the infected quarter soon after the 12th day, later in the blood

serum and later still in the other quarters of the mammary gland. Using goats, similar results were obtained with Newcastle virus, and removal of the mammary gland after the detection of neutralizing antibodies in the circulation resulted in a sharp drop in titre.

Batty & Warrack (1955), using a technique elaborated by Oakley and his colleagues (Oakley, Batty & Warrack 1951, 1955; Oakley, Warrack & Batty 1949, 1954), were able to demonstrate antibody production in the secretions and tissue extracts from the mammary gland of the rabbit. The intrinsic controls of this method are absent from much of the other work presented in support of local antibody production. For example, when the circulating titre is higher than that of muco-antibody, a relatively higher titre in the particular portion of the mammary gland injected with antigen compared with the rest of the gland may only be a reflexion of altered tissue permeability and does not per se provide evidence for local antibody production. Therefore it is usually necessary to demonstrate the earlier appearance of muco-antibody compared with circulating antibody or to show a higher local antibody titre.

Oakley's technique involves the simultaneous stimulation of circulating antibodies to two antigens. One of these antigens is subsequently injected into the site at which the production of local antibody is to be determined. Some ten days later the animal is bled out, tissue extracts are prepared from the appropriate organs, and the secretions where these are available are collected. The ratios of the circulating antibody titres to the two antigens at the time of death are compared with those present in the tissue extracts or secretions. An antibody ratio significantly higher in the tissue extract than in the serum presents evidence in favour of local antibody production. The method is particularly valuable in controlling inflammatory effects and other factors capable of producing local changes in tissue permeability.

Extensive studies have been made on the antibodies to Br. abortus in the milk from infected cattle since they have diagnostic significance, however, it is also apparent that protective vaccination with the live Br. abortus Strain 19 produces antibodies in the whey (Marr & Williams, 1958; Kerr, Pearson & Rankin, 1958). A consideration of the possible origin of these antibodies serves to introduce a specialized function of the mammary gland associated with the formation of colostrum.

It is well known that the mammary gland secretion of many species is modified at or about the time of parturition and reflects then a higher concentration of those antibodies present in the maternal serum.

Blakemore & Garner (1956) and Garner & Crawley (1958) have shown that following the intravenous injection of homologous gamma-globulin to a cow approaching calving, the antibody was concentrated in the udder to at least 13 times the blood concentration. This is reflected by a drop in serum gamma-globulin as the normal cow or heifer approaches calving (Pierce, 1955; Larson & Kendall, 1957). The humoral origin of the antibody in the colostrum is also supported by the results obtained by Larson & Gillespie (1957), using radioactive carbon. Although certain milk proteins appeared to be synthesized in the cow's udder, the C^{14} incorporated into the immune lactoglobulin (the colostrum fraction containing antibody activity) suggested that these proteins entered the milk preformed

from the circulation. Recently Kerr, Pearson & Rankin (1958) have made an important observation on the origin of antibodies at certain periods in milk. Towards the end of the normal bovine lactation but while a cow may still be giving two gallons of milk a day antibody derived from the circulation begins to appear in the milk. These muco-antibodies together with those accumulating in the udder during the weeks immediately prior to calving are a reflexion of a specialized function of the mammary gland of great intrinsic interest although a complication in the study of local antibody formation by the udder.

Campbell, Porter & Petersen (1950) have drawn attention to the proliferation of plasma cells in the bovine udder before and during calving and their disappearance 18 days after calving; similarly if milking was discontinued there was evidence of plasmacytosis which subsided 15 days after milking was recommenced. In view of the association of plasma cells with antibody, the observations are offered in support of local antibody formation.

REPRODUCTIVE TRACT. The diagnostic significance of muco-antibodies recovered from the reproductive tract of cattle has stimulated widespread veterinary interest, and the three major infective diseases causing infertility (trichomoniasis, vibriosis and brucellosis) have been extensively investigated.

Trichomonas foetus is a protozoon parasitic in the reproductive tract of cattle; living and reproducing in the mucous secretions, it is non-invasive and ideally fulfils the requirements for studies on the production of local antibodies and on the significance of muco-antibodies. Technically a satisfactory serological test is available for the detection of serum agglutinins (Robertson, 1941; Kerr & Robertson, 1941, 1943; Pierce, 1947 a). However, specific serum antibody was detected only when the natural infection provided an adequate antigenic stimulus, e.g. in cases of abortion or pyometra. Serological tests on known infected cattle showed a maximum of only 13% positive reactions (Pierce, 1949 a).

Byrne & Nelson (1939), studying experimental trichomoniasis in the reproductive tract of rabbits, demonstrated an acquired resistance to re-infection which was not necessarily coincidental with specific circulating antibody. Since the humoral antibody which followed the intravenous injection of antigen did not confer any immunity, the authors considered that the acquired resistance following natural infection was local in origin. Florent (1941) noted that vaginal mucin from infected cattle agglutinated and immobilized Tr. foetus, and in 1946(b) Pierce showed the specificity of the agglutinins for Tr. foetus and Br. abortus and devised a method for preparing extracts of vaginal mucin suitable for agglutination tests. Since over 60% of infected animals gave positive mucin reactions the diagnostic value of the test was established (Pierce, 1947 b, 1949 b), and Florent (1947, 1948, 1949), using a slightly different technique, obtained similar results. Vaginal muco-antibodies could be detected in cattle without circulating antibody (Pierce, 1947 b; Florent, 1948) while the subcutaneous, intramuscular and intravenous injection of antigen produced circulating but not muco-antibodies (Florent, 1948; Kerr & Robertson, 1953; Pierce, 1953). However, Kerr & Robertson (1947) showed that dead

antigen instilled into the bovine uterus provoked muco-antibody in the vaginal and uterine secretions and demonstrated the presence of uterine muco-antibody following natural infection with trichomoniasis. The inability of humoral antibodies (vide supra) to gain access to the site of infection within the lumen of the reproductive tract explains the doubtful success following attempts to convey either a passive immunity by intravenous injection of immune serum (Garlick, Bartlett & Hammond, 1944) or an active immunity by intramuscular vaccination (Kerr & Robertson, 1946). Nevertheless Bartlett, Hammond & Garlick (1944) and Kerr & Robertson (1947) were able to show the acquisition of some resistance following natural infection, i.e., by the local application of antigen.

Kerr & Robertson (1953) sensitized the bovine uterus actively by repeated instillation of antigen, and passively by intra-uterine injection of homologous immune serum. However, anaphylaxis could not be produced to a primary intra-uterine injection of antigen into animals with a high circulating titre evoked by previous intramuscular injections of antigen. Therefore humoral antibody did not appear to become fixed in the uterine tissues in the same way as antibody formed as the result of local infection. (See also self-cure phenomenon in helminth infections, page 22).

These observations show the fallacy in the assumption that humoral antibody distributes itself throughout the tissues. The barrier to the transmission of humoral antibody to the mucous surface also suggests a probable local origin for the muco-antibodies within the reproductive tract. Unlike many examples of muco-antibody those at the mucous surface of the bovine vagina and uterus as the result of natural infection, are frequently present in the absence of circulating antibody or appear earlier and are of higher titre.

Pierce (1946a) drew attention to the changed morphology of Tr. foetus from about the 66th day of infection; the organisms became rounded, the anterior flagella swathed and the movement of the undulating membrane slow or intermittent and very difficult to detect microscopically in discharges; this change was subsequently associated with the presence of muco-antibody at low titre in the vaginal mucin (Pierce, 1947 b). At high muco-antibody titres organisms were not recovered. However, re-infection of the vagina could occur via the cervix from the persisting infection of the uterus. Provided the relaxation of the cervix was temporary the vagina again rapidly eliminated the organism. Therefore it is apparent first, that muco-antibody is effective in vivo and secondly that immunologically the bovine vagina and uterus may behave independently (Pierce, 1950).

Uterine muco-antibodies are apparently less persistent than those of the vagina and Kerr & Robertson (1953) have tentatively suggested that this may be related to the cyclical mucosal destruction associated with oestrus. Since vaginal muco-antibody is diluted out during the oestral mucous flow this combination of factors may account for the relatively short-lived immunity to trichomoniasis observed in the field.

Stegenga & Terpstra (1949) successfully adapted the trichomonas vaginal mucin agglutination test to the diagnosis of infection of the bovine reproductive tract with Vibrio fetus (see also Sjollema, 1950; Stegenga,

1950 and Terpstra & Eisma, 1951). Again the detection of muco-antibody was superior to serum antibody for diagnosis. These authors noted the independence of muco- and serum agglutinins and this was confirmed by Lawson & MacKinnon (1952).

Kerr (1955) studied the vaginal and uterine muco-antibodies to Brucella abortus and again showed the barrier to the passage of humoral antibodies on to the surface of the mucous membrane of the bovine reproductive tract. Muco-agglutinins were absent from animals with circulating titres as high as 1:2,560 resulting from the intramuscular injection of antigen. Conversely intra-uterine instillation of killed Br. abortus Strain 19 vaccine resulted in a maximum vaginal muco-antibody titre of 1:1,280, uterine muco-antibody 1:20, and serum titre of 1:10. When live Strain 19 vaccine was instilled, vaginal titres of 1:20,480, uterine 1:1,280 and serum 1:140 were obtained. Thus unlike trichomonas antigen, very little circulating antibody was stimulated by the local instillation of brucella antigen (see Kerr & Robertson, 1943). This could be related to a difference in solubility between the two antigens, to the manner of their absorption from the lumen of the uterus and therefore the sites of antibody formation stimulated. Kerr, Pearson & Rankin (1958) have investigated the status of brucellosis in 147 herds by the combined use of agglutination tests on serum, whey and vaginal mucin and by guinea-pig inoculation. Routine immunization with Strain 19 vaccine severely limited the diagnostic significance of serum titres, but muco-antibodies were not present as the result of vaccination. During the later stages of lactation circulating antibodies were secreted by the udder so that whey titres at certain times could also be misleading. Therefore, in the absence of extravasated blood, the positive vaginal muco-agglutination test was a reliable indication of a virulent infection. The use of vaginal muco-antibodies has also been investigated for the diagnosis of brucellosis by Jepsen & Vindekilde (1951), Romváry (1955), Roepke et al. (1957) and Marr & Williams (1958).

Batty & Warrack (1955), using Oakley's technique, have also shown the local production of antibody in the vagina and uterus of the rabbit. Since the ratio of the antibodies in the tissue extract was similar to that in the mucin, there was a strong indication for the local origin of the muco-antibodies.

A cellular basis for the origin of local muco-antibody has not been extensively studied. Plasma cells appear to be intimately concerned with antibody production or concentration and Brudenell (1955) found a heavy infiltration of these cells in acute salpingitis of the human endometrium.

A virgin heifer in which muco-antibodies were elicited by the repeated vaginal instillation of dead Br. abortus vaccine showed approximately four times as many plasma cells in the vaginal mucosa compared with a control heifer. A limited number of cells frequently in the most superficial areas of the mucous membrane and similar to Russell bodies were also observed (Pierce, 1958). These cells have been associated by fluorescence techniques with local concentration of antibody (see page 22) and may provide one method whereby antibody could be passed on to the mucous surface by a non-vascular route.

Local infections of the genital tract of the bull with Br. abortus also

produced local antibody titres in the seminal plasma in excess of the circulating titre (Bendixen & Blom, 1947; Christensen, 1948). Plasma cell infiltration was a characteristic of these infections (Christensen, 1948).

RESPIRATORY TRACT. Bull & McKee (1929) and Walsh & Cannon (1936) found an increase in the local and humoral resistance of rabbits to virulent pneumococci after immunization by intranasal instillation of heat-killed pneumococci. Further work on the effect of the intranasal administration of bacterial antigens by Walsh, Sullivan & Cannon (1932), Cannon & Walsh (1937) and Walsh & Cannon (1938) showed that higher titres were associated with those tissues proximal to the site of antigen inoculation. The passive immunity resulting from antiserum sprayed intranasally has also been studied (Evans, 1944; North, 1946).

Using the technique of Burnet & Timmins (1937), Gray (1947) demonstrated a greater degree of protection in mice following intranasal rather than subcutaneous injection of Haemophilus pertussis vaccine and Cooper (1952), using intracerebral challenge as a test for humoral immunity, showed that this followed the intraperitoneal but not intranasal inoculation of mice with pertussis vaccine. Intranasal immunization, however, was very effective against intranasal challenge. This work suggests that immunity may be local rather than humoral. However, North & Anderson (1942) showed that whereas the intranasal instillation of H. pertussis provided a more effective immunity in mice than that following intraperitoneal or subcutaneous injection, the effect was non-specific and could equally well be achieved following similar histological changes induced by the intranasal instillation of Escherichia coli.

These observations have not differentiated muco-antibodies from those in tissue extracts, however Francis (1940) and Fazekas de St. Groth & Donnelley (1950 a) emphasized the fundamental importance of muco-antibodies compared with circulating antibodies in studies where the pathogen is an obligatory respiratory parasite, approaching and attacking susceptible cells from their mucosal surface and without previously entering the bloodstream. Certain viruses fall into this category and at the same time fulfil the ideal requirements for experimental studies on local antibody formation.

The site of antibody formation following intranasal instillation of influenza virus into mice was shown to be the mediastinal lymph nodes (Burnet & Lush, 1938). However, Burnet, Lush & Jackson (1939) also showed anti-viral activity in human nasal secretions. Further studies by Francis (1940), Francis & Brightman (1941), Francis, Pearson, Sullivan & Brown (1943) and Francis (1943) showed the antibody nature of the virus-inhibiting properties of human nasal secretion.

Fazekas de St. Groth and his colleagues have made an extensive study of influenza virus infection in mice with particular reference to the origin of the bronchial muco-antibodies. Fazekas de St. Groth & Donnelley (1950a) showed that infection initiated by intranasal instillation of influenza virus into mice stimulated antibodies which appeared in the bronchial mucin at the same time as the serum. The coincidence of appearance of antibody in serum and mucin is in conformity with the observations of

Francis et al. (1943) in human subjects vaccinated subcutaneously with live or inactivated virus, moreover fluctuations in the two titres were parallel. These observations are at variance with those on the reproductive tract where antibody did not pass readily from the circulation on to the mucous surface, and also with those on the gut where, following infection per os, copro-antibody titres appeared earlier than circulating antibodies.

Fazekas de St. Groth & Donnelley (1950 a) showed that approximately five units of muco-anti-haemagglutinin were found in the bronchial mucin for every 100 units in the circulation after instilling virus intranasally, while animals immunized by the intraperitoneal or subcutaneous injection of virus secreted only 0.4 units to every 100 in the serum. Fazekas de St. Groth & Donnelley (1950 b) further showed that whereas there was a correlation between circulating antibody and resistance to infection the real correlation was between resistance and the antibody concentration at the mucous surface. Fazekas de St. Groth (1950, 1951), Fazekas de St. Groth & Donnelley (1950 c), and Fazekas de St. Groth, Donnelley & Graham (1951) further investigated the distribution of antibody between the serum and bronchial mucin. They showed that the low local antibody to humoral antibody ratio (0.4/100) following intraperitoneal immunization could be raised to 5.0/100 by the simultaneous nasal administration of a heterologous virus. This phenomenon is described as "pathotopic potentiation" and agents capable of producing it are pathotopic adjuvants, thus distinguishing them from the adjuvants which, when mixed with vaccines, enhance the immune response. Pathotopic adjuvants were shown to act by increasing capillary and/or tissue permeability by virtue of a mild inflammatory change, (i.e., in the absence of any demarcating fibrinous blockade). Examples of these adjuvants are influenza vaccines, meta-periodate ion and certain astringents.

Fazekas de St. Groth & Graham (1954) showed that when mice were passively immunized by the parenteral injection of homologous serum about 1/300th of the antibodies injected reached the respiratory mucous surface compared with only 1/2,000th when heterologous serum was used. This is an important observation and raises the problem of antibody transport across membranes.

The practical importance may also be considerable, for example in the treatment of local infections at a mucous surface homologous rather than heterologous antiserum may be more effective.

The experimental results show the importance of bronchial muco-antibodies in resistance to infection and give an example of a muco-antibody which is derived from extravasated humoral antibody. Moreover, although local immunization with vaccine potentiated the antibody in favour of the respiratory tract, the circulating titre was considerably lower than that produced by the intraperitoneal injection of similar amounts of vaccine, to an extent which offset the advantage of the local potentiation. Local immunization therefore is not indicated although Fazekas de St. Groth (1950) has suggested the possible use of pathotopic adjuvants to make more serum antibody available to the effective site in emergencies such as epidemics.

Whether, following local infection, the upper respiratory passages

also derive muco-antibody from the circulation or more resemble the reproductive tract in forming local antibody is not apparent. In the earlier experiments an acquired resistance was demonstrated following local immunization, but the relative significance of the upper and lower respiratory tract was not investigated. Further difficulties arise following the rapid clearance of antigen from the upper respiratory passages, much is lost, and most of it quickly passes into the lungs by aspiration. The particular characteristics of a pathogen must also be considered; whether, for example, the organism merely gains entrance via the nose and passes benignly through the upper respiratory passages, multiplying and producing an effective antigenic stimulus in the lungs. In this virus infection extravasated humoral antibody was the significant factor, while infections localized to the upper respiratory tract, sinuses etc., may stimulate local muco-antibody and be relatively uninfluenced by circulating antibody.

URINARY TRACT. Burrows (1948) and Burrows & Havens (1948) actively immunized guinea-pigs and man intraperitoneally with cholera vaccine. Antibody was detected in the urine and their results again suggested local production of antibody since in common with copro-antibody, urinary muco-antibodies showed an earlier rise, peak and decline in titre as compared with serum antibody. Guinea-pigs immunized passively by intraperitoneal injection of homologous and heterologous antiserum also excreted antibody in the urine. The antibody having been inoculated into the peritoneum, the route whereby it reached the urine was speculative; however the circulatory system seemed the least likely as the antibody was detected in the urine before it was detected in the blood. Active cellular transport by fixed cells or perhaps lymphocytes, or by a passive transfer through extra-cellular spaces are possibilities and could be approached experimentally, by comparative studies using the intravenous and intraperitoneal injection of immune serum.

Contrasting results in man were obtained by Naylor & Caldwell (1953), who were unable to detect muco-antibodies in the urine following immunization with T.A.B. vaccine. This discrepancy could be related to a different distribution of the antigen. Very good evidence in support of the local production of urinary muco-antibody was provided by further studies on urinary enteric carriers. These patients did not have gross injury to the urinary tract and showed muco-antibody specific for the infecting organism; the ratio of urinary titre to serum titre was between 1:5–1:20. The carriers were actively immunized by the subcutaneous injection of an unrelated Salmonella and a grossly disproportionate ratio (1:80–1:160) between urine and serum was obtained. It would appear therefore that pathotopic potentiation was not a significant factor.

Further work by Archer, Bangham, Dunbar & Ritchie (1950), Archer, Goffe & Ritchie (1952) and Archer & Miller (1952) showed the presence of urinary muco-antibodies in man homologous with the organism recovered from urinary carriers of Salmonella. Evidence for the local production of antibody was complicated by superimposed infections of the bladder with Schistosoma, however, the urinary titres were sufficiently high compared with those in the plasma to show that they could not be attributed to

haemorrhage. Some evidence in favour of local antibody production was shown by the higher titre and more regular recovery of antibodies to the carried organism (S. typhi) in a patient with circulating antibody at the same titre to three salmonella namely S. typhi, paratyphi A and B.

Studies on Leptospira icterohaemorrhagiae and L. canicola in man, dog and rat by van der Hoeden (1936) revealed specific agglutinins in the urine. They were not demonstrated until a few days after the appearance of agglutinins in the blood and although they were always of lower titre they persisted for a longer time after recovery. Injection of killed organisms in normal subjects stimulated circulating antibodies, but not urinary muco-antibodies; this, together with the persistence of muco-agglutinins long after recovery when any inflammatory alteration in the permeability of the bladder had subsided, again suggests local antibody production.

DISCUSSION. Specific muco-antibodies against bacteria, virus and protozoa, but not against helminths, have been demonstrated at the surface of mucous membranes. Whether they are local, derived from sites in the immediate vicinity, or humoral (as defined) has usually been determined indirectly by deductive reasoning from measurements of the relative antibody concentrations in the serum and mucin when related to time. Frequently the muco-antibody titre does not exceed the serum titre; when it does, as for example in studies on the reproductive tract, there is stronger evidence for local production. However, where important deductions are made from the comparisons of titres, insufficient attention has been given to the possible effect on end-point of the differing milieux of serum and mucin in in vitro tests.

Oakley's method provides a valuable technique, with intrinsic control of many factors, for the study of local antibody concentrations which may without unreasonable assumptions be related to antibody formation. The method is particularly useful in studies where muco-antibody titres do not exceed serum titres, for controlling the effect of local changes in tissue permeability, and for example would control the normal physiological antibody concentrating activity of the udder.

In contradistinction to muco-antibody demonstrated in the urine, faeces and secretions from the udder and genital tract for which in certain instances a local origin is indicated, Fazekas de St. Groth and his colleagues have shown the humoral origin of bronchial muco-antibody. This may be related to the unusually intimate relationship between the capillary network of the alveoli and the mucous surface. Whether muco-antibodies derived from the mucous membrane of the upper respiratory tract have a similar origin is uncertain and a comparative study of the two sites has not been made.

The work of Fazekas de St. Groth et al. emphasizes the importance of correctly assessing the origin of muco-antibodies following the local application of an antigen. A rise in muco-antibody titre may be the reflexion of local antibody production, or the extravasation of humoral antibody arising from a local alteration in tissue permeability.

The udder must be considered as a special case, local production of antibody seems probable and also pathotopic potentiation resulting from

mastitis. In addition there is the special physiological ability of the gland to secrete humoral antibody in the milk towards the end of lactation (Kerr, Pearson & Rankin, 1958) and to concentrate it to a remarkable extent in the precolostrum during the weeks immediately prior to calving. In considering evidence for the local production of antibody this property has been assumed to be peculiar to the udder.

Evidence has been presented which suggests that antibody given by intraperitoneal injection may reach the mucous surface of the gut and perhaps the bladder by a non-vascular route. Whether muco-antibodies would be equally readily detected if the immune serum were given intravenously is equivocal and is an important aspect requiring further study. Therefore any assumption regarding the availability of humoral antibody arising from studies on muco-antibody or immunity at a mucous surface following intraperitoneal passive immunization may be incorrect.

Active and passive immunization may induce circulating antibody which has little significance for some infections and only indirect significance for other infections localized at mucous surfaces. For example in experimental virus influenza in mice the resistance is directly proportional to the muco-antibody titre and although this was humoral in origin, the serum antibody titre does not give an accurate measure of resistance.

The results of comparative studies on the serum and mucin titres also show the error in assuming a distribution of antibody between the plasma and mucous surfaces. In several instances an effective barrier to humoral antibody has been demonstrated, and vaginal mucin may be without activity in animals with high circulating titres resulting from intramuscular antigen injections.

Recent work has shown the association of local concentrations of antibody with plasma cells and Russell bodies, both of which have been described infiltrating the mucous membrane following local infection or local application of antigen. However, further work is required to show the manner in which local antibody is passed on to the mucous surface. Research is also required into the mechanism involved when active or passive humoral antibody, or that given passively by the intraperitoneal route is passed on to the mucous surface and whether this is an active or passive process.

Further study is required into the relative facility with which antibodies in homologous and heterologous antisera administered passively reach a mucous surface since this may have considerable practical significance in the treatment of localized infections at these sites.

Muco-antibodies have been shown to be of primary importance in the defence against characteristic types of infection, usually non-invasive and confined at a mucous surface or in the superficial mucosa. They may have a diagnostic significance superior to the serum antibodies. Circulating antibody has been shown to be unreliable as an index of resistance to infection at a mucous surface. Orthodox immunization procedures, stimulating humoral antibody, may not confer a local immunity as these methods are not equally effective in producing muco-antibody.

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The Pneumonias of the Sheep with special reference to Enzootic Pneumonia and Jaagsiekte

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A report on the diseases of sheep issued by the British Veterinary Association (1955), lists ten major diseases of sheep, which, on a conservative estimate, cause an annual loss of over one million pounds sterling to the sheep industry in the U.K. The pneumonias of sheep are not included in that list.

In a recent review of the respiratory diseases of sheep, Stevens (1957) classes pneumonia of the sheep "as one of the most important clinical syndromes in sheep, both as an acute killing disease and in a more chronic form where loss is less apparent". He found an incidence of 15.9% in 400 consecutive autopsies on sheep in England, a figure which tallies with that noted by Pounden et al. (1956) in apparently healthy sheep at an abattoir in the U.S.A. Whether these groups of sheep were affected with a specific type or types of pneumonia liable to appear spontaneously at any time in sheep flocks was not determined; that pneumonia is a more frequent entity than hitherto suspected is an important observation. It is well established that in practically all countries where sheep farming is practised, losses from specific pneumonia are a steady drain on the economy, even if they tend to be somewhat sporadic and seasonal, and vary in intensity from year to year. In addition, non-specific terminal pneumonia is frequently noted in a variety of diseases to which the sheep is subject. It would appear that the sheep is peculiarly liable to succumb to secondary pneumonia of this type.

Many species of bacteria have been isolated from the pneumonic lesions, but whether they are commensal and normally found in healthy sheep lung, has not been adequately investigated.

Lungworm infestations of sheep may involve 90% of a flock and it is apparent that the sheep lung can withstand considerable tissue damage by a variety of nematode parasites. Some of these have their normal habitat in the lung for varying periods of time, others pass part of their life cycle in the lung. Bronchiolitis with a nodular pneumonia is a common sequel, as is catarrhal exudation into alveoli with a varying degree of involvement of vascular channels, which would afford a favourable pabulum for the propagation of potential bacterial pathogens if such are normally resident in the lung. That sheep can tolerate nematode invasion of the lung with little ill effect, despite considerable damage to the lung, is an argument against the residence within the lung tissues of potentially dangerous organisms. As will be shown later in the present review, however, it is well established that nematode invasion of the sheep lung can lead to grievous loss from secondary bacterial infection.

This review is intended to indicate clearly that sheep pneumonia is a disease of serious consequence to sheep farming and that it is of world-wide distribution. Certain specific forms of the disease exist. The literature on these is somewhat meagre and often contradictory. The first impression gained from a survey of the literature would seem to be that under certain conditions of stress and strain a few animals in a flock are prone to succumb rapidly to pneumonia and then, when environmental conditions improve, losses cease. This is not the whole story. In Iceland, a specific type of sheep pneumonia -- jaagsiekte -- was introduced by the importation of two Karakul rams from Germany. This was followed a few years later by devastating outbreaks of the same disease which necessitated a slaughter policy to eradicate. Over one million sheep were slaughtered. The disease is said not to have reappeared since this tragic episode but, as will be shown in this review, it has not been eliminated; what apparently has happened is that locally bred sheep have become somewhat less susceptible to an infection that was at first overwhelming in the local sheep population. There are two strange anomalies. First, the literature shows little evidence of the same disease in Germany. Secondly, one of the earliest records of the existence of this type of pneumonia of sheep comes from Scotland (M'Fadyean, 1894). In recent years, it has been shown to have a limited incidence in sheep flocks both in Scotland and England, fortunately with little tendency to spread to adjacent flocks. The same disease has been the cause of grave concern in S. Africa and widely distant Peru but is quite unknown in the great sheep ranching countries of Australia and New Zealand. It has a local distribution in the U. S. A. In Kenya, a country with a sheep-rearing potential equal to that of England, the same disease wrecked early efforts at sheep farming. It is known in Scandanavian countries and, possibly in France, where it has, however, occasioned little anxiety. The literature on this important disease is somewhat meagre. There are more references to a disease termed enzootic sheep pneumonia, a disease which, though more widespread and perhaps better understood, is certainly less devastating.

This review is concerned with an analysis of the literature on both these diseases which have an established place in veterinary pathology. Research on the problem of other sheep pneumonias has progressed over the last decade and the review includes new knowledge and present progress.

ENZOOTIC SHEEP PNEUMONIA

The disease has practically a world-wide distribution. Comprehensive accounts of it are given by a number of workers in many countries, viz. in Great Britain (Jowett, 1930: Leyshon, 1932: Montgomerie et al., 1938: Campbell et al., 1949: Stamp et al., 1955); in America (Spray 1923: Newsom & Cross, 1932: Marsh, 1923b, 1938, 1953); in Germany (Miessner & Köser, 1931: Oppermann, 1936); in Iceland (Dungal, 1931); in Norway (Woxholtt et al., (1952); in Switzerland (Dolder & Leuenberger, 1948); in France (Gourdon et al., 1957: Delpy, 1932); in Australia (Beveridge, 1937); in New Zealand (Salisbury, 1957; Downey, 1957) and in South Africa (Henning & Brown, 1936). In all these countries, the incidence and

clinical features follow a similar pattern, though with slight variations. The pathological details vary considerably in the accounts given by different workers. Descriptions of the aetiology of the disease, with particular reference to bacteriological studies, present a confused picture. Indecision and contradiction beset an analysis of the various attempts by different workers to prove the transmissibility of a disease of standard pattern to healthy sheep.

Incidence. Leyshon observed a high incidence of pneumonia in two flocks of sheep, with a mortality of 7%. The majority of the sheep developed a chronic pneumonia, as a sequel to an acute sudden attack, which killed some sheep within a few hours. Jowett found pneumonia with pleurisy in healthy slaughter sheep aged six months and over. Campbell et al. record an outbreak in housed sheep in Scotland; 5 out of 24 animals were affected and two died. Montgomerie et al. describe the disease in North Wales. Adult sheep were mainly affected, though the disease did occur in lambs. Predisposing factors were a sudden change in weather, particularly associated with close penning, crowding, housing, dipping or transit by road or rail. Morbidity was very high and mortality about 15%. Stamp et al. describe a disease in lambs and hoggets in the Border area of Scotland, which was septicaemic and not primarily pneumonic. Losses were heavy, 10–20% of the flock, and a predisposing cause was a change of pasture in the late summer. The environmental factors probably determined the onset of the disease which could be stopped in its course by a change of pasture. Newsom & Cross reported the loss of 116 of 3,000 lambs, from pneumonia which occurred 18 days after shipping. Marsh (1953) found a high mortality resulting from pneumonia in lambs within the first few months of life; in one outbreak 92 of 533 lambs died. A similar disease affecting lambs was described in Germany by Miessner & Köser and by Oppermann, who considered that it could only attack sheep or lambs debilitated by transport or by being driven along dirty tracks or fed dirty hay. Dungal describes the disease as one of housed sheep. Beveridge states that in two groups of five lambs three and a half months old, three died very suddenly from pneumonia.

Salisbury records that, between 1936–37, 6,000 sheep from 118 flocks died from a form of pneumonia similar to that described by Montgomerie. Adult sheep were implicated in the outbreaks, which occurred from October to January, when cases ceased spontaneously. Death was usually sudden but some cases lingered for 2–3 days. Recoveries were rare. Salisbury states that, in 1949, approximately 3.5% of all sheep on ten properties succumbed to the same disease. The disease coincided with luxuriant pasture growth and most of the serious losses occurred after shearing. Downey, working independently, gives a similar history of the disease. Henning & Brown mention a virulent attack of pneumonia confined to Ryeland sheep and not spreading to Merinos. Three rams died.

These are the only workers who have commented at all upon the history and incidence of the disease. That many of them comment on predisposing factors as governing the incidence of the disease seems important. The symptoms of the disease are generally dealt with briefly.

All agree that the disease is sudden in onset and rapid death the rule. The outward evidence of the disease is some coughing, nasal discharge, variable fever, rapid loss of condition and prostration. Most observers have concentrated upon descriptions of the bacteriology and aetiology. Accounts of the pathology are somewhat meagre.

Pathology. In Leyshon's cases, the right apical lobe of the lung was chiefly involved, often the mediastinal lobe and the lower borders of the other lobes. The hepatized lung contained greyish nodules, about 1 cm. in diameter, scattered through the pulp. No parasites were demonstrable. Slight pleurisy with a small quantity of clear, straw-coloured exudate in the chest cavity was noted. Jowett describes the same distribution of the pneumonic lesion, with a sharp line of demarcation between diseased and healthy lung, a more pronounced fibrinous pleurisy and infiltration of the lung stroma with the same type of exudate as found in the chest cavity. In more advanced cases, lung abscess was usual and a purulent pleurisy with firm adhesions. Montgomerie describes early degenerative change in liver and kidney, in addition to extensive pneumonia in both lungs. The chest cavity contained clear exudate and petechiae on the pleural surface and heart membranes. Fibrinous pleurisy was seen only in subacute and chronic cases. The histology showed bronchopneumonia. The early lesion consisted of scattered haemorrhages within groups of alveoli, with fibres in the alveolar spaces, scanty cellular reaction and some infiltration of interstitial tissue and cuffing of bronchioles.

Campbell et al. found consolidation of one apical lobe and a few pneumonic patches scattered through the remaining lobes. Degenerative changes were seen in the kidneys and petechiae on the heart membranes.

The description of the disease given by Stamp et al. was rather that of a septicaemia than of a pneumonia. The trachea and bronchi showed intense congestion and contained blood-stained froth. A clear, blood-tinged exudate was present in the pleural cavity and the pleural surfaces were dull in colour without pleurisy. The lung tissue contained scattered haemorrhages and a frank pneumonia was seen only in cases surviving for a few days. There was an intense oedema of interlobular septa and the associated lymph nodes were swollen and oedematous. Microscopically, lung tissue showed an acute distension of capillaries and venules and marked oedema of the alveoli and septal tissue. The capillaries contained bacterial emboli. The liver showed acute hepatitis or degenerative fatty changes with bacterial embolism. The kidneys showed lesions somewhat resembling those seen in pulpy kidney disease. Areas of haemorrhage with marginal oedema were present on the pyloric surface of the abomasum and, sometimes, on the folds of the omasum or on the duodenal mucosa. Ecchymoses were noted within the heart membranes. Marsh (1953) described two forms of the pathology. The first was a true septicaemia without pneumonia, the lesions very closely resembling those described by Stamp. The second was an uncomplicated bronchopneumonia. Delpy described lesions of bronchopneumonia as insidious in development with an acute enteritis.

The pathology of the disease described by Dungal corresponds

closely with that given by Montgomerie. Salisbury described severe fibrinous pleurisy with loose adhesions between lung and chest wall, apical lobes completely consolidated with a fibrinous type pneumonia, tending to extend to the right cardiac lobe, marked interstitial oedema and haemorrhages into the alveolar tissue with early foci of necrosis associated with bacterial embolism. Ecchymoses within the heart membranes were conspicuous, as was a haemorrhagic tracheitis. The liver was somewhat congested and the kidneys more so, with prominent streaky haemorrhages, particularly in the medulla. Downey gave a somewhat different account of the pathology. The pleural cavity contained a variable amount of clear, straw-coloured fluid exudate. Fibrinous pleurisy was noted. The lungs showed a generalized congestion and oedema and, when this was extensive, it was difficult to see the areas of consolidation. When these were present they were bluish-red in colour, raised above the level of the surrounding tissue and were found chiefly on the dorsal surface of the lung. The lesion cut like glandular tissue and the cut surface had a characteristic appearance, with alternating areas of grey and bluish-red consolidation. The histological picture was intense interstitial oedema, haemorrhage around and into groups of alveoli and catarrhal bronchiolitis; the alveoli were distended and plugged with exudate consisting mainly of large mononuclear cells. Neutrophiles were inconspicuous or absent.

Henning & Brown described a necrotic, fibrinous pleuropneumonia with exudate in the pleural and pericardial sacs, severe tracheitis, some hepatitis and splenitis, and degenerative changes in the kidney.

The diversity of lesions described in this disease might be interpreted as one of degree and not of kind. All tend to follow a basic pattern but are subject to considerable variation, determined by the intensity of the infection or, conversely, by the susceptibility of the host to the infection, particularly at times when resistance is lowered by untoward influences of weather and other factors. It could as well be argued that there is little likelihood of a common aetiology, because in most animal diseases, even those of sheep, there is a fairly standard pathological pattern upon which a pathological diagnosis of the disease can reasonably be made. It would appear to be a safe deduction that, unless a specific common infection can be authenticated for all the pathological variations now enumerated, it would be premature and rash to classify enzootic pneumonia of sheep as a specific disease entity in all countries where it has been described. Stamp et al., perhaps impressed with the finding of kidney lesions suggestive of pulpy kidney disease, examined the possibility of this disease as an explanation of the pathological syndrome, in which lesions were as dominant without, as within, the thoracic cavity. Salisbury commented on the fact that the sudden deaths in the disease investigated by him were associated with lush pastures and that deaths suddenly ceased when, it would appear, sheep were no longer exposed to this environmental risk.

Bacteriology. Most workers on this disease have found a pasteurella-like organism plentiful or swarming, usually to the exclusion of all other types of bacteria, in films made from the pneumonic lung.

Pure cultures of this organism can easily be grown in ordinary broth or on the surface of agar plates. Leyshon isolated this type of organism in culture from pleural fluid, lung, associated lymph node and blood: the organism was typically bipolar in tissues and culture.

Jowett isolated a similar organism in pure culture from early lesions of the disease but in more advanced cases Corynebacterium pyogenes, also, was isolated, and sometimes Fusiformis necrophorus and streptococci. Some workers have isolated the organism only from the pneumonic lesions or the pleural exudate, when present. Others have isolated it from blood as well as from the lung (Campbell, Spray). Henning & Brown obtained pure cultures from lung and liver and no growth from blood. Leyshon cultured the organism from lung, pleural fluid, mediastinal lymph node and blood. Stamp isolated the same type of organism from lung, liver, kidney and spleen. Dungal also isolated it from these tissues, as well as from the mediastinal lymph node.

Newsom & Cross and also Henning isolated two different types of pasteurella from the same type of pneumonia. Marsh found the organism in only 75% of 92 lambs examined. Spray isolated three main types of a pasteurella-like organism from 56 cases of enzootic pneumonia in lambs; these were described as types S.8, S.3 and S.14. Some workers have described the organism as seen in lung films and cultures as ovoid and bipolar: others have described strictly bacillary forms. Opinion is divided on capsule formation. On the essential cultural characters of the various strains isolated, there is general agreement. In broth, uniform turbidity is produced within 24 hours; in older cultures, pellicle formation may be seen, with a viscid deposit at the bottom of the tube, the broth then clearing slightly. Cultures on agar plates show colonies resembling typical pasteurella and growth is enhanced by blood or serum. Cultures die within 2-3 days. The difference of opinion on the intrinsic nature of the organisms isolated is aggravated when the biochemical characters of the cultures are examined. Very few strains are comparable on such grounds.

Of 24 strains biochemically examined by Montgomerie, acid was produced (almost constantly) in glucose, maltose, saccharose, laevulose, mannite and dextrin: glycerol was usually fermented and about one half attacked xylose. All strains were slightly haemolytic. Growth on MacConkey and the indole reaction were not stated. All the strains examined by stamp except three produced acid in glucose, saccharose and mannitol and in laevulose, maltose and sorbitol. Only one strain reacted with inulin and, in a wide range of other sugars tested, reactions were quite variable. Indole reaction was negative and there was fair growth on MacConkey: no mention was made of haemolysis. Of the strains examined by Dungal, all gave a uniform biochemical reaction, viz. acid in glucose, laevulose, galactose, mannite, saccharose, sorbite, xylose and maltose: no haemolysis on blood agar plates, indole negative and good growth on MacConkey: organism not capsulated. The strains isolated by Newsom & Cross fell into two well-defined groups on the basis of haemolysis on blood agar, indole production and rabbit pathogenicity. For those strains that were haemolytic, indole-negative and not pathogenic for the rabbit, the name Past. haemolytica was proposed.

Gourdon et al. examined 17 strains of a pasteurella-like organism isolated from sheep with pneumonia and from the respiratory tract of healthy sheep. Carbohydrate reactions were exceedingly variable but practically constant for each strain. All strains were non-haemolytic and nine produced indole. Similarly, the three main types of pasteurella isolated from cases of enzootic sheep pneumonia by Spray exhibited variable carbohydrate reactions which were, however, specific for each type. Type S. 8 was non-haemolytic in blood agar and produced indole but it could not be considered a true pasteurella, as it attacked none of the sugars usually attacked by strains of Past. septica.

Rosenbusch & Merchant (1939) examined 44 strains of an organism conforming to the pasteurella type, of which 13 were from sheep. The sheep strains could be divided into four groups. Groups 1–3 were non-haemolytic on blood agar, indole-positive and pathogenic for mice. Group 4 corresponded to Jones Group 1 and was haemolytic on blood agar, indole-negative and non-pathogenic for mice. Strains of Groups 1–3 could be further differentiated by fermentation reactions.

The literature yields further examples of the astonishing variability on bacteriological criteria of the types of pasteurella-like organisms which have been isolated by different workers from outbreaks of enzootic sheep pneumonia, a variability that leads to a suspicion that the various strains have only one feature in common, that is their appearance in tissues and exudates, when their pathogenesis for sheep and laboratory animals is examined. Thus, Montgomerie found that his cultures inoculated by intravenous, intraperitoneal and subcutaneous routes in rabbits, guinea-pigs, mice and pigeons produced no disease, except in mice when they were given very large doses by the intraperitoneal route. His strains were also non-pathogenic for sheep, by the intravenous and subcutaneous routes.

Stamp et al. reproduced the disease in 7 to 9-month-old lambs by intravenous inoculation, either of broth cultures or lung suspensions, death occurring in about 12 hours. Subcutaneous inoculation was almost equally successful, except that some lambs developed cellulitis instead of the typical syndrome. Intratracheal inoculation did not set up disease. Further, these materials retained their pathogenicity when stored for some months at -60°C. No account is given of the pathogenicity of the strains for laboratory animals. The strains isolated by Campbell et al. were pathogenic in high doses for mice, by the intraperitoneal route but non-pathogenic for rabbits and guinea-pigs. Dungal's strains reacted similarly in small animals but reproduced the disease in sheep when inoculated intratracheally. Of the two types isolated by Newsom & Cross, one killed rabbits, the other did not. All three types studied by Spray killed rabbits and guinea-pigs but only two killed mice.

Miessner & Köser describe strains pathogenic for sheep and guinea-pigs but not for other laboratory animals. These workers constantly isolated a strain of Escherichia coli along with pasteurella-like organisms: the E. coli strain was pathogenic in sheep. Gourdon et al. found 12 out of 17 strains pathogenic for guinea-pigs and non-pathogenic for sheep and the usual range of small animals. The virulent strain isolated by Henning killed sheep and guinea-pigs but not rabbits. The strains

isolated by Beveridge killed neither mice nor rabbits and were doubtfully pathogenic in guinea-pigs. Newsom & Cross inoculated 34 rabbits with lung suspension prepared from an equal number of affected lambs and 32 died within 24 hours: an organism defined as Past. oviseptica was isolated from the heart blood of rabbits that succumbed to the inoculation. Further elaboration of the variability of the pathogenicity of organisms isolated by various workers is needless: all observations emphasize the point.

Serology. Jones (1921) examined a number of strains of pasteurella from cattle and sheep. Sera were prepared in rabbits against these strains which, on the basis of agglutination tests, were divided into four groups. The sheep strains were classified as Jones' Group I. In the same way, Montgomerie serologically classified his sheep strains into two groups. An authentic pasteurella of avian origin was not agglutinated by Group I and II sera but a strain of the organism isolated by Dungal from pneumonia in sheep was proved identical with Montgomerie's serotype II. Newsom & Cross likewise divided the pasteurella-like strains isolated from sheep pneumonia into two serological groups. Spray showed that his Types S. 3 and S. 14 were serologically related but that Type S. 8 belonged to a different serological group. Rosenbusch & Merchant serologically classified their biochemical Groups I–IV. Strains of Groups I–III could be serologically grouped together, though some cross agglutination occurred with strains, which did not permit of exact serological differentiation; Group IV exhibited no serological link with Groups I–III.

Bosworth & Lovell (1944) examined serologically 55 strains of pasteurella-like organisms isolated either from the nasal mucus of sheep during an outbreak of nasal catarrh and coughing following inclement weather, or from the nasal cavity or lower portion of the trachea of healthy sheep immediately after slaughter. All the strains were classified as 'haemolytic cocco-bacilli' and conformed with the description given by Jones of Pasteurella haemolytica (Jones' Group I). Sera were prepared in rabbits against two of these strains (all of which, incidentally, gave exceedingly variable reactions in a wide range of sugars), as well as against the strains studied by Montgomerie et al. and classified as their sub-groups I and II. The clear-cut results obtained by these authors were not obtained by Bosworth & Lovell, as some cross agglutination occurred, but it was possible to show the serological affinity of 36 of the 55 strains examined; 24 appeared to fall into their sub-group I, one into their sub-group II, 11 gave cross agglutination with Groups I and II sera, and 19 strains showed no agglutination with either of the sera.

Francis (1941) isolated 5 strains of pasteurella-like organisms from the tonsils of a number of healthy sheep. These conformed biochemically and serologically to Montgomerie's serotype I.

The finding of Bosworth & Lovell, and of Francis, that organisms of the types described can frequently be isolated from the respiratory tract of perfectly healthy sheep has been confirmed by Campbell et al. and equally denied by other workers. Newsom & Cross found the organism only once in bronchial scrapings from 92 healthy slaughter sheep.

Stevens (1957) found the organism in the trachea of only one of 18 sheep dead from diseases other than enzootic pneumonia, and never isolated it from the lungs of sheep that had died from jaagsiekte. On the other hand, Marsh (1953) isolated pasteurella-like organisms quite constantly from the lesions of jaagsiekte, in the aetiology of which they are not concerned.

Dungal states that enzootic pneumonia is spread by carrier sheep from which the organism is infrequently isolated. This must be considered a generalization in face of the fact that many different types of a pasteurella-like organism can be isolated from sheep affected with this disease. Much work is obviously still required to substantiate the hypothesis that a few sheep in a flock may harbour a potential pathogen of this nature which, under certain conditions, may assume a degree of virulence sufficient to cause an outbreak of enzootic sheep pneumonia. It is difficult to believe that each of the various types and strains of this organism described by various workers has, within itself, the capacity to generate a specific clinical syndrome, in which even the pathological findings are by no means constant. This would be a novel conception of an older hypothesis that a particular disease syndrome is constantly associated with a specific type of infection.

Marsh (1953) states that the organisms which have been isolated from the lesions of the disease are merely secondary invaders. That so many different bacterial types should occur would then be regarded as a freak finding, somewhat unique in the annals of veterinary pathology. Perhaps Montgomerie et al. are of the same opinion when they state that it has yet to be determined whether a virus is the direct cause of the disease.

The Search for a Virus. Montgomerie failed to reproduce the disease in sheep with cultures; he then examined the infectivity of lung tissues selected from typical clinical cases. The clear supernatant fluid, obtained after centrifuging ground-up suspension of the very early lesion, failed to produce the disease in sheep by intranasal or by intratracheal inoculation but a combination of intratracheal and intravenous, or the use of such routes along with the intrapulmonary, killed sheep rapidly. On P. M. examination the pleural cavity was filled with sero-fibrinous exudate; the interstitial tissue of the lung was widely infiltrated with the same exudate. No pasteurella organisms were isolated from the lung. It proved impossible to establish the infective agent by serial passage in sheep. Rabbits and ferrets could not be infected with the same inoculum insufflated under ether anaesthesia or by direct inoculation into the lung. In mice, however, intranasal insufflation set up a specific type of pneumonia in 3 of 12 mice. Berkefeld V filtrates of the lung of infected mice reproduced the same disease. Ten passages of the apparent virus were made in mice. Unfortunately, the development of ectromelia in later passage mice confused the issue. When sheep were intranasally insufflated with mouse lung suspension, no illness developed. When, however, sheep were given the same inoculation, along with the pasteurella type organism isolated from natural cases of enzootic pneumonia, they developed a type of pneumonia quite typical of this disease. Further efforts in transmission of the experimentally produced

disease failed.

This work has been confirmed to some extent by Downey (1957), who isolated from suspensions of sheep lung, after treatment with antibiotics, an apparent virus which grew on the chorio-allantoic membrane of the chick embryo. This virus, in combination with the pasteurella type organism isolated from the sheep lung, reproduced the disease in 2 of 10 sheep inoculated by intravenous and intratracheal routes; the same virus was re-isolated on the egg membrane from the sheep that reacted but failed to infect in further sheep passages.

Immunity. The majority of workers who have investigated this aspect of the disease consider that some degree of immunity can be conferred on sheep by vaccines prepared from the pasteurella type strains isolated. Dungal used a phenolized vaccine on a large scale with considerable success. Woxholtt; Dolder & Leuenberger; also Miessner & Köser record the same experience. The last-named workers also found an immune serum prepared in sheep, both with the pasteurella type organism and a strain of *E. coli* constantly isolated along with this organism, to be of practical value. Montgomerie found vaccines ineffective. Henning & Brown failed to immunize guinea-pigs with a vaccine prepared from the virulent strain of the organism isolated by them.

OTHER TYPES OF SHEEP PNEUMONIA

Several workers have described other types of sheep pneumonia. The general pattern of some of these types corresponds quite closely on clinical and pathological grounds with the better-recognized enzootic pneumonia of sheep, which has been described.

Morcos *et al.* (1946) described a contagious pneumonia of sheep in the Sudan. A virus was isolated from blood and internal organs of clinical cases. This proved pathogenic for rabbits but not guinea-pigs nor mice (there is no mention of pathogenicity for sheep). A pasteurella type organism was isolated from the lung of affected sheep, often along with *C. pyogenes*, but both organisms were regarded as secondary invaders. No details were given of the methods of isolation of the virus or of its physical properties. It was claimed that a formolized vaccine prepared from the liver and spleen of affected sheep gave good results in control of the disease.

Pokorný (1955) isolated a virus in embryonated eggs inoculated with lung suspension from one of several lambs affected with pneumonia. The third egg passage when instilled intranasally in two lambs reproduced the typical disease. Pasteurella-like organisms were sometimes isolated from the lungs of affected lambs and were considered secondary invaders.

Caminopetros (1949) described outbreaks of a virulent type of pneumonia in sheep in the Balkan States. The disease spread rapidly to adjacent flocks, to goats and to the human population in neighbouring countries. The cause of this serious disease was determined to be *Rickettsia burneti* (Q fever).

Grini & Koppang (1956) described a form of pneumonia of both sheep and goats, considered to be caused by a streptococcus belonging to

Lancefield's Group C. This organism was constantly isolated from the lesions of bronchopneumonia.

Marsh (1938) described a form of pneumonia in adult sheep which may be responsible for heavy losses. The exciting cause was the lungworm Protostrongylus stilesi: secondary bacterial invasion of the damaged lung is caused by C. pyogenes and a species of pasteurella.

In Canada, 'lunger disease' of range sheep has proved to be a secondary pneumonia following poisoning by the plant Astragalus campestris (Bruce, 1934).

Heikkila (1956) studied a highly infectious pleuropneumonia of sheep in Angola and isolated from affected lungs a member of the pleuropneumonia group of organisms which was held to be the cause of the disease.

Ramirez et al. (1951) isolated an apparent virus from the lungs of pneumonic sheep. This was maintained on the chorio-allantoic membrane of developing chick embryos for 19 passages, with lesions in the embryonic lung.

McKercher (1952) isolated from lungs of pneumonic sheep a virus of the psittacosis–lymphogranuloma–pneumonitis group of viruses. This virus was not serologically identified with the virus of enzootic abortion of sheep.

In Palestine, Gilbert (1937) showed that sheep may be seriously affected with a form of bronchopneumonia with pleurisy spreading from affected goats.

Pfeifferella whitmori has been isolated from a disease of sheep in Queensland in which the symptoms were coughing and lameness (Cottew, 1950; Queensland report, 1951).

Roberts (1957) isolated C. equi from bronchopneumonia of sheep in Australia. Abscess formation was usual in affected lungs.

PULMONARY TUBERCULOSIS OF THE SHEEP

Ovine tuberculosis is a rare disease in Gt. Britain, Griffith (1925), quoted an incidence of 5 cases in about one and one third million sheep examined in abattoirs. In the U.S.A. 16 cases were detected in 96 million slaughtered sheep and lambs (Harshfield & Roderick, 1934). In Germany, during the years 1904–1928, the disease was seen in 0.2–0.22% of all slaughter sheep (Hutyra et al.). M'Fadyean (quoted by Griffith) suggested that the higher incidence in Germany was probably due to sheep being housed for long periods of the year. The first case recorded in the literature was a lamb that had been reared on milk from a tuberculous cow (Bollinger, 1873). The first authentic account of the disease in sheep in Britain was given by M'Fadyean (1900, 1902), two cases being recorded. Foulerton (1902) described generalized tuberculosis in one sheep seen at Smithfield and quotes M'Fadyean as stating that the rareness of the disease is due not to a natural immunity but to the open air life. Griffith described two cases from which the avian type tubercle bacillus was isolated and this would appear to be the first instance of an accurate typing of the causal organism. Jowett (1928) isolated the bovine type from two tuberculous sheep at Edinburgh, and emphasized the danger of feeding

bovine milk to motherless lambs.

In the two cases described by M'Fadyean, one was the side of a carcass in which the parietal pleura showed nodular lesions of tuberculosis. In the second case the lung was largely replaced by caseous and partially calcified material, the diseased areas varying in size from that of a pea to that of a pigeon's egg. The broncho-mediastinal lymph nodes were affected. In Foulerton's case, clusters of grape-like lesions were seen on both pleural surfaces and both lungs had numerous tubercles and extensive consolidation; the disease was generalized. Jowett described nodular lesions about one cm. in diameter scattered throughout both lungs. An extensive tuberculous granuloma involved the base and upper border of the right lung. Two large lesions each about the size of a hen's egg and consisting of fibro-caseo-calcareous material were present in the left lung, in addition to numerous tubercles. The broncho-mediastinal lymph nodes showed gross lesions.

Griffith described the production of pulmonary tuberculosis in two lambs subcutaneously inoculated with culture of bovine type isolated from a sheep. Murphy (1935) reported the first case of ovine tuberculosis in Ireland: both lungs contained caseo-calcareous lesions up to the size of a marble, and the associated lymph nodes were grossly enlarged and calcified. Davidov (quoted by Carmichael, 1938) emphasized the appearance of the broncho-mediastinal lymph nodes in ovine tuberculosis, and records a case where these attained a weight of 562 g. Harshfield & Roderick examined six cases, and the lesions were similar, particularly with regard to early and progressive calcification of lesions. Carmichael quotes Petit, Mayer, Nieberle & Eber in support of the observation that resistance of sheep to tuberculosis is associated with the high degree of calcification which accompanies tuberculous lesions. He described 12 cases in indigenous Persian-type sheep in Uganda, 11 of which were caused by bovine and one by human type tubercle bacillus. The lungs of three sheep showed very severe and advanced lesions with much cavitation and bronchiectasis: one case showed, in addition, acute bronchitis and tracheitis with plugs of muco-purulent or muco-caseous material swarming with tubercle bacilli: the associated lymph nodes were inflamed and contained caseous material. However, in the remaining cases the lesions showed the same tendency to early calcification as in sheep in temperate climates; the human type of bacillus was isolated from a case showing cavitation.

Francis (1958) has annotated the pathological findings in 44 cases of ovine tuberculosis and 36 of these showed pulmonary involvement; 24 were caused by the avian, 19 by the bovine and 1 by the human type tubercle bacillus.

Luke (1958) has reviewed the literature on the disease and gives an account of the types of infection encountered by various workers and experiments undertaken by them to show the susceptibility of sheep and lambs to the three types of the tubercle bacillus.

JAAGSIEKTE AND SIMILAR FORMS OF PNEUMONIA OF SHEEP

Eber (1899) first recorded the existence in Germany of a chronic

pneumonia of sheep, which had no tendency to resolution and in which epithelial aberration of lung tissue was a constant feature. We owe the first account of this new disease of sheep, which has since gained importance in veterinary pathology, to Robertson (1904), who in S. Africa named it "jaagsiekte" from the Afrikaans, jaagen — to drive, siekte — sickness. It had been known since 1893 to farmers in the Cape Province and Robertson quotes Hutcheon as having observed cases even earlier. Mitchell (1915), also in S. Africa, finally established that it is a specific contagious pneumonia of sheep and de Kock (1929) added to the knowledge of the disease in that country and considered it to be a malignant pulmonary affection of sheep. Marsh (1923a) recorded similar chronic progressive pneumonia of sheep in the U. S. A. known as Montana sheep disease. Dungal et al. (1938) described a form of chronic pneumonia of sheep in Iceland, which they called pulmonary adenomatosis. Still later, Sigurdsson (1954) encountered in Iceland yet another type of chronic pneumonia of sheep, which he considered different from both jaagsiekte and the disease described by Dungal et al. This disease was called maedi, meaning dyspnoea. In 1888, M'Fadyean had incriminated lungworms as a cause of chronic pneumonia of sheep in Scotland (M'Fadyean, 1894). In 1938, however, he revised this view and stated that some of the sheep lungs originally examined showed undoubted lesions of jaagsiekte. This must be accepted as the first record of the disease in Gt. Britain, although Blakemore & Bosworth (1941) provided the first accurate description of the disease there. They were followed by Harbour & Jamieson (1946) and Stevens (1957).

Creech & Gochenour (1936) recorded a disease seemingly identical with jaagsiekte in the north-western regions of the U. S. A. Véliez (1945) (personal communication) described the same disease in Peru. Pattison (1946) found undoubted lesions of jaagsiekte in the lung of a sheep in Palestine.

Maedi was introduced into Iceland along with jaagsiekte, but was not considered to be the same disease. Jaagsiekte has over recent years ravaged sheep flocks in Kenya (Shirlaw, 1956). In France, a form of chronic pneumonia of sheep, known as "bouhite", is confined to certain parts of the country. It is said to resemble jaagsiekte in general character, but to show little real relationship. Bos (1951) stated that a disease similar to jaagsiekte existed in sheep in the Netherlands.

Incidence and Epizootiology. Although jaagsiekte and similar diseases are known in many countries, only in two countries have they assumed epizootic proportion. Mitchell described jaagsiekte as a form of pneumonia in sheep in the cold, high districts of Cape Colony. On clinical grounds it appeared infectious, spreading particularly during wet seasons. It was later diagnosed in the Orange Free State and in the Transvaal, but probably had an even wider distribution in the Union of South Africa. Sheep about three years of age were chiefly affected and all breeds proved susceptible. The disease spread rapidly in sheep kept in kraals, where the infection was maintained for long periods. Annual losses amounted to 10–20%, and in some flocks much exceeded this figure. The incidence was much less in range sheep

and, in them, the contagion spread slowly. It was observed to be introduced into clean flocks by sub-clinical cases of the disease.

The misfortune that overtook sheep farmers in S. Africa during the early part of the century was repeated to a greater extent in Iceland about two decades later. Pulmonary adenomatosis was apparently introduced into Iceland by two Karakul rams imported from Germany in 1933. Three years later, it struck sheep flocks in Iceland as a major epizootic and accounted for losses of 50% or more amongst flocks. The disease showed no sign of abatement during the following years. Dungal et al. (1938), in a survey of affected farms, showed that it spread from diseased to healthy flocks and that it was highly contagious. Close housing in the winter months seemed to provide ideal conditions for its insidious spread.

It was realized in 1939 that maedi was rampant in Iceland, causing between 1939–1952 the death of 150,000 sheep. An annual mortality of 10–20% on many farms with no sign of a gradual drop in incidence, necessitated a slaughter policy (1944–1952), which involved over 300,000 sheep. Since re-stocking, the disease is said not to have reappeared in Iceland, but Sigurdsson regards it as too early yet to conclude that the steps taken have completed its eradication, particularly as it is essentially insidious in onset. Sigurdsson does not comment upon the fact that jaagsiekte and maedi largely overlapped in incidence during the course of the epizootic. Pálsson (1948), referring to this catastrophe in Iceland, noted that the Aldabol breed of sheep was little affected and believed that it might prove possible to breed resistant strains.

Véliez (personal communication, 1954) stated that the annual mortality from jaagsiekte in Peru was about 1.5% of all flocks. In a survey of the sheep pneumonias in Kenya, Shirlaw (1956) found this disease widespread throughout sheep farms, with up to 30% annual mortality in affected flocks. The folding of sheep at night to protect them from predators helped in the rapid spread of the disease, which was seen, though infrequently, in lambs as young as three months.

Bouhite somewhat resembles Montana sheep disease described by Marsh. It has been suggested that sheep grazing pastures afforested with pine trees are particularly prone to it, though Guilhon & Chauvier (1952) described the disease on pastures where pine trees were absent. Lucam (1945b) considered bouhite to be a malignant disease of the lung and termed it "malignant pulmonary lymphomatosis".

Great Britain has been little affected with jaagsiekte, though M'Fadyean stated that the disease had been known since 1894 and maintained that there was little hope of its having since disappeared. The meagre incidence in Gt. Britain, as in Germany, is a peculiar phenomenon, especially since the worst epizootic ever recorded (in Iceland) spread from Germany. In Gt. Britain, half a century elapsed before the disease was again described by Blakemore & Bosworth, who found it in a flock of sheep in Norfolk to which had recently been added 100 cross-bred ewes from Scotland; a few cases of the disease occurred amongst the latter without spread to the main flock. A few years later, Harbour & Jamieson described the disease in two flocks in Scotland, in both of which it had apparently existed unrecognized for a number of years. Stevens (1957) quotes Loosmore as having recently diagnosed it in the Reading area and

Fincham in S. W. England. He also described an outbreak in Norfolk introduced by sheep from Scotland. During the previous three years, annual losses had been about 8%. Ewes 3–4 years old were affected, but a case was also detected in one aged ten months.

It appears, then, that the various forms of chronic pneumonia described follow a common pattern in incidence and epizootiology, even to the age of the sheep affected. The variation in the incidence and severity of jaagsiekte-like disease in the several countries where the disease has appeared seems inexplicable. Duran-Reynals et al. (1958) tentatively offer a possible explanation when they state "the disease may have begun a natural decline in incidence in affected countries, possibly showing the declination of a long-term epidemic curve". If to this statement they had added the possibility of variation in the virulence of an infective agent, this could be accepted as a reason for the paucity of the disease in Britain and other countries where the disease is known to exist and its tragic significance in other countries such as Iceland. However, this statement would postulate the existence of the disease in sheep for a very long time prior to its discovery.

Clinical Features. According to Mitchell and other workers, the earliest sign is an increase in respiration rate, even when the sheep is at rest, with severe respiratory distress when it is driven. The course is marked by progressive debility, anaemia, emaciation and loss of wool. Deaths occur after 2–8 months' illness but some cases survive for a year or longer, especially if nursed. Some may appear to recover temporarily but the disease is invariably fatal. It is easily diagnosed by holding the sheep up by the hind legs, when a copious exudate pours from the nostrils. Dungal et al. state, however, that loss of condition is seen only in the last stage of the disease, when sheep may die suddenly from a terminal bacterial pneumonia; alternatively, sudden death, without pneumonia, may occur when sheep are over-driven or even handled. Sigurdsson describes maedi in similar terms, though he states that it must be distinguished from jaagsiekte on clinical grounds. However, the only clinical difference would seem to be that sheep affected with maedi do not evacuate fluid from the nostrils. Bouhite and Montana sheep disease both follow the clinical pattern of jaagsiekte.

Pathology. Mitchell gives a classic account of the lesions. He states that the earliest lesion is a marble-sized nodule projecting slightly above the lung surface, usually situated in the cardiac lobe, fleshy in consistency and hyperaemic in appearance. The cut surface shows tiny, greyish, translucent foci of millet-seed size, which infiltrate the surrounding hyperaemic lung tissue. Early pleurisy with adhesion is noted. The nodule slowly increases in size and progressively invades the lung tissue, which becomes atelectatic; compensatory emphysema occurs in more distant parts of the lung, especially in the apical lobes. Slow fibrosis of the nodule then develops and the central part retracts below the surrounding level, following cicatrization of the new fibroblastic tissue. At this stage, the lesion cuts like cartilage and the cut surface is white, glistening and avascular. The openings of the smaller bronchi are

occluded by the fibrotic change. The broncho-mediastinal lymph nodes are swollen and oedematous.

Histological examination reveals the earliest lesion as a haemorrhagic area with alveoli packed with erythrocytes and soon increasingly infiltrated with lymphocyte type cells and macrophages, while the central zone is invaded by fibroblasts. On the periphery of the mass, a combination of all types of cell is seen widely infiltrating into the surrounding stroma. The round cells fan out through the interlobular connective tissue obliterating the alveolar structure and the histology of the newly-formed tissue resembles that of a lympho-sarcoma. Discrete lymphoid nodules develop within the gradually encroaching cellular tissue and, with this progressive extension, entire lobes of the lung are involved. "Cuffing" of small bronchi and blood vessels with lymphocytes and the formation of nodules on the thickened walls of the small bronchi is a usual feature. The mucous membrane of the latter is thickened and polypoid outgrowths of the epithelium are seen. The ultimate lesion is a dense mass of fibrous tissue involving whole lobes. Dungal et al. describe the pathology of pulmonary adenomatosis of the sheep in fundamentally similar terms. At post-mortem examination, the lung is found grossly enlarged and does not collapse. The apical and middle lobes are mainly affected and slowly become transformed into a huge, tumour-like, fibrotic mass, somewhat water-logged. In the smaller bronchi, considerable epithelial proliferation is seen. This is exaggerated in the terminal bronchioles and alveolar tissues, with the formation of islands or larger areas of adenomatous tissue. Myxomatous degeneration may occur in the main mass of new fibrotic tissue with occasional giant cells. Cell mitosis is never seen in the adenomatous tissue.

The description of the pathology of maedi given by Sigurdsson is essentially similar to that seen in jaagsiekte, save that the entire lung is spongy rather than fibrotic. The lung stroma is diffusely infiltrated with lymphocytes, macrophages, large mononuclear cells and fibroblasts. Accumulations of lymphocytes are very prominent, particularly around the bronchi, which are invariably thickened. Epithelial proliferation of the bronchioles is noted. The large mononuclear cells when stained by Giemsa stain show intracytoplasmic inclusion bodies, either single or multiple.

Cowdry (1925) examined the lung tissues of 38 apparently healthy slaughter sheep in S. Africa and compared the histology of these with an equal number obtained from districts in the U. S. A., where the disease is known not to exist. Thirty-three of the S. African lungs showed the epithelial proliferation considered characteristic of jaagsiekte. Areas of thickening were focal in distribution and developed from cellular infiltration and proliferation of existing interstitial tissue. His description of the essential pathology of the disease does not differ from that given by Mitchell. He suggested that the epithelial proliferations were the result of the stimulus given by the infiltration of new cellular tissue, which latter is the predominant and persistent feature of jaagsiekte. He pointed out that epithelial metaplasia of the lung is often seen in a variety of disease conditions other than jaagsiekte, viz. plant poisonings, nematode infections, chronic bacterial infections and in influenza and other virus

infections. He considered it probable on the grounds of the pathology that jaagsiekte is of virus aetiology.

Perhaps the best instance of the tendency of lung epithelium to proliferate under the stimulus of a phytotoxin is seen in jaagsiekte of the horse which is a sporadic affection caused by the ingestion of the toxic weed Crotalaria spp. (Theiler, 1918). Roux (1903) observed lesions of pulmonary adenomatosis in cases of sheep pox. M'Fadyean (1938) stated that the lesions of jaagsiekte and of Montana sheep disease are fundamentally similar and are caused by a chronic inflammatory process, possibly due to a virus. Cowdry and Marsh supported this opinion. Marsh (personal communication, 1957) states that the various forms of the jaagsiekte-complex in sheep are quite comparable on pathological grounds. The pathology of bouhite is very similar to that of Montana sheep disease, except that adenomatous metaplasia accompanies cellular infiltration of stroma and is often, in fact, the dominant feature of the disease. The cellular infiltration is often so dense that the term malignant pulmonary lymphomatosis has been given to the disease. Aynaud et al. (1932) have described adenomatous metastasis to the mediastinal lymph nodes and to the subcutaneous lumbar lymphatic tissue in two cases.

Aetiology. Duran-Reynals et al. group the various forms of sheep pneumonia enumerated in the pulmonary adenomatosis complex in three categories. (1) At least some, and probably all, forms are infectious; (2) members of the complex range in pathology from inflammatory or infiltrative to carcinomatous; (3) various forms whilst almost certainly of viral aetiology can occur in both epizootic and enzootic forms, thus resembling both the picture of classic viral transmission (epizootic) and that of natural tumour epidemiology (occurring at low rates, or enzootic). More than one type of virus may be involved in the pathological processes.

Though M'Fadyean found extensive lungworm infection in the lungs of sheep with lesions that simulated those of jaagsiekte, and though Cowdry hints at the possibility of a nematode infection in, perhaps, some cases, a special investigation carried out by Dungal et al. into the association of the lungworm, Muellerius capillaris, with the disease led to the conclusion that it is very improbable that the lungworm has any connexion with causation, or development or rate of mortality resulting from jaagsiekte.

There is no doubt that under certain conditions and in certain countries the jaagsiekte group of diseases is highly infectious. Ample clinical evidence exists (Mitchell; Dungal; Sigurdsson). In this connexion, it should be noted that, in Iceland, deaths from terminal pneumonia were very frequent both in pulmonary adenomatosis and maedi and that the organism styled Past. haemolytica was usually isolated from the affected lungs. It may well be, therefore, that enzootic sheep pneumonia (which Dungal had already described in Iceland) complicated the issue. In S. Africa, where losses were also heavy, Cowdry observed similar bacterial infection within the lung tissues of a number of sheep examined. In Kenya, Shirlaw (1954) found abscess formation frequent in the lungs of

sheep affected with jaagsiekte. A coliform organism was uniformly isolated from the pus and equally from the lesions of terminal bronchopneumonia. This organism was highly pathogenic in sheep, causing a severe and often fatal cellulitis on subcutaneous inoculation. Neither Marsh nor Stevens found Past. haemolytica in lungs examined bacteriologically, though Stevens found abscesses in 4 of 17 cases, from which streptococci and coliform organisms were isolated: neither in England nor the U.S.A. has jaagsiekte-like disease been epizootic.

Various workers have attempted to prove a virus aetiology using local sheep; this, despite the observation of Cowdry in S. Africa, that in an enzootic area many sheep exhibit incipient lesions of the disease. Obviously, experimental evidence must be suspect unless known clean sheep have been used.

Dungal et al. inoculated three lambs into the lung, each with 2.0 ml. of saline suspension of lung emulsion from a typical case of pulmonary adenomatosis. Eight months later, one developed clinical signs of the disease, which was confirmed on autopsy. Whether there was any evidence of incipient disease in the lungs of the remaining lambs was not, apparently, examined. Five sheep were each inoculated in the same way with Seitz filtrate of the same lung emulsion and none showed lesions of the disease, when examined post mortem eight months later.

In Sigurdsson's experiments, which were carried out under conditions of strict isolation, with lambs from known maedi-free districts, two groups of five lambs were kept in contact with cases of maedi for a period of three months; one group of five lambs was exposed by adding faeces to the drinking water; a fourth group was inoculated intravenously, intranasally and directly into the lung with lung suspension from a typical maedi case. Three groups of five lambs were kept as controls under the same conditions of isolation. All were killed after 30 months. None had shown any sign of the clinical disease. It was found that the average weight of lungs in animals of the experimental groups was statistically higher than the average weight of lungs from the control animals. The lungs from the former groups showed the specific lesions of the disease on microscopic examination. In a modified repetition of this experiment, lambs were killed at varying intervals and specific lung lesions were found 30 days after inoculation. Three different haemagglutinating substances were isolated from the lungs of sheep affected with maedi, all active against fowl erythrocytes. Two, however, were discarded from further study, as they were also isolated from the lungs of healthy sheep. Blood sera from maedi cases did not neutralize the third. Extracts of such lung tissue prepared in various ways failed to fix complement with serum from diseased sheep. Commenting on this work, Duran-Reynals et al. state "unfortunately, no general method for the demonstration of specific antigen in the lungs of diseased animals is available, so that it has proved impossible to determine whether serologic relationships exist between geographically separated forms of the disease, nor has it proved possible to cultivate the infective agent of maedi" — presumably by inoculation of chick embryos with lung material, though this is not stated, nor does the literature contain any evidence of such experiments having been attempted, until Shirlaw (1955, 1956) isolated in chick embryos a

virus from the lungs of sheep affected with jaagsiekte. The titration end-point of this virus was 10^{-7}, and at the end of 1956 it had successfully undergone 80 egg passages, killing the embryo at 3–4 days after inoculation. Specific lesions of a virus type of pneumonitis with epithelial involvement could be evoked in known healthy sheep (from an area where the disease was known never to have occurred) when egg-adapted virus was inoculated directly into the lung. Identical lesions could be produced with a Seitz E K filtrate of lung emulsion from a natural, early case of the disease. The egg-adapted virus could not be passaged in mice by insufflation, nor by intraperitoneal, intracerebral or intrathoracic routes, and suspensions of infected lung likewise failed in mice when inoculated by the same routes. He also showed (1957) that complement-fixing antibodies against an antigen prepared from the yolk sac membranes of virus infected eggs were present in the sera of sheep affected with jaagsiekte. An antigen prepared in the same way from the yolk sac membrane of normal eggs did not fix complement, in the presence of the same sheep sera.

Dungal (1946) transmitted pulmonary lymphomatosis to healthy sheep with a gradocol membrane filtrate (0.9μ) of suspension of diseased sheep lung given by the intranasal route. Marsh (personal communication, 1957) stated that he had killed and examined a sheep inoculated four years previously with filtrate prepared from the lung of a "lunger" sheep and that he had now reproduced the disease several times with the same inoculum.

Immunity. On sheep farms in Kenya, selected because of a past history of serious losses from jaagsiekte, a formolized vaccine prepared from the lungs of diseased sheep has been put to a five-year trial now ending. This vaccine has been used in successive lamb crops and, wherever it has been used, the incidence of the disease has dwindled to less than 1% as against a former incidence of 30% or even more. During the period of trial, large numbers of chronic "lungers" were present in the flocks to which vaccinated lambs were added at weaning, so that ample evidence of resistance to a constant infection existing over the years was obtained. During this time, the chronic "lungers" were gradually eliminated by the disease.

Cuba-Caparó (see Duran-Reynals et al., 1958) states that this type of vaccine has proved effective in control of the disease in Peru, which is the first confirmation from any other country of the worth of the vaccine tried in Kenya.

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